Institute of Metabolic Science

MRC | Epidemiology Unit

Genetic Analysis of Complex Traits

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About the title

- Complex traits refer to common diseases or traits with no clear modes of Mendelian inheritance Reduced penetrance, heterogeneity, phenocopy, pleiotrophy,… (Lander & Schork 1994), environmental factors, examples include diabetes, heart diseases, mental disorders, height, body-mass index (BMI)
- Methods include the assessment of familial aggregation for heritability, identification of major gene effect, study of cosegregation of genetic marker with putative disease-predisposing loci in the so-called linkage studies and association studies in search of frequency differences between cases and controls and/or correlation between genotype and phenotype as a quantitative trait. Morton et al. (1983), Khoury et al. (1993), Thomas (2004)

A sketch

- We provide an overview of genetic analysis of complex traits in humans in the context of large volume of genetic data.
- While there are many analytical issues, our focus is more on the practical side.
- We provide specific examples of genetic association study.
- We are not limited to R, and would provide examples using systems other than R whenever appropriate.
- Our hope remains to be that this will serve as a forum for a range of issues and a contact point for future researches.
- Questions are welcome during the sessions.

Contents

- The presentation consists of four parts:
  I. Overview
  II. Analytic tools and association testing
  III. Miscellaneous topics
  IV. OpenMx and NCBI2R
  V. Conclusion
- You may find materials from useR!2008 and useR!2009 tutorials relevant. They are both available from my personal home page.

What have changed?

- We are quite far with topics in both useR!2008 and useR!2009, esp. genome-wide association studies (GWAS) of directly genotyped and imputed SNPs and interaction analysis. It is routine with Stata function which automating analysis by SNPTEST. We have updated results regarding genetic predisposition score from the EPIC-Norfolk study. We have a better understanding of the SNP annotation, via UCSC/galaxy and in particular NCBI2R. There are other changes, e.g., functions MiMa has been replaced with metafor package.
- I am more proficient with R and have consolidated R/gap functions fbsize, pbsize, ccsize, and added ab and masize. We have explored 'raw' storage mode in R which is central to snpMatrix and GenABEL.
- We provide further examples. We add examples for chromosome X data, and obtained imputed genotypes for analysis. We also add materials regarding OpenMx. In the future, more materials on Bioconductor can be added.

Monographs

- Morton NE. Rao DC, Lalouel JM. Methods in Genetic Epidemiology. Karger, 1983.
- Khoury MJ, Beaty TH, Cohen BH. Fundamentals of Genetic Epidemiology. Oxford University Press, 1993.
- Falconer DS, Mackay TFC. Introduction to Quantitative Genetics, 4e. Longman, 1996
- Hartl D, Clark AG. Principles of Population Genetics, 3e. Sinauer Associates, Inc. 1997
- Lange K. Mathematical and Statistical Methods for Genetics Analysis. 2e, Springer 2002
- Sorensen D, Gianola D. Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics. Springer 2002
- Thomas DC. Statistical Methods in Genetic Epidemiology, Oxford University Press, 2004
- Armitage P, Colton T (Eds). Encyclopedia of Biostistics, 2e, Wiley, 2005

Monographs

- Elston RC, Johnson WD. Basic Biostatistics for Geneticists and Epidemiologists, A Practical Approach. Wiley, 2005
- Ahrens W, Pigeot I (Eds). Handbook of Epidemiology. Springer, 2005
- Balding DJ, Bishop M, Cannings C (Eds). Handbook of Statistical Genetics, 3e, Wiley, 2007
- Siegmund D, Yakir B. The Statistics of Gene Mapping. Springer 2007
- Wu R, Ma C-X, Casella G. Statistical Genetics of Quantitative Traits-Linkage, Maps and QTL. Springer, 2007
- Speicher MR, Antonarakis SE, Motulsky AG. Vogel and Motulsky's Human Genetics: Problems and Approaches, 4e, Springer, 2010
- Lin S, H Zhao (Eds). Handbook on Analyzing Human Genetic Data: Computational Approaches and Software. Springer, 2010

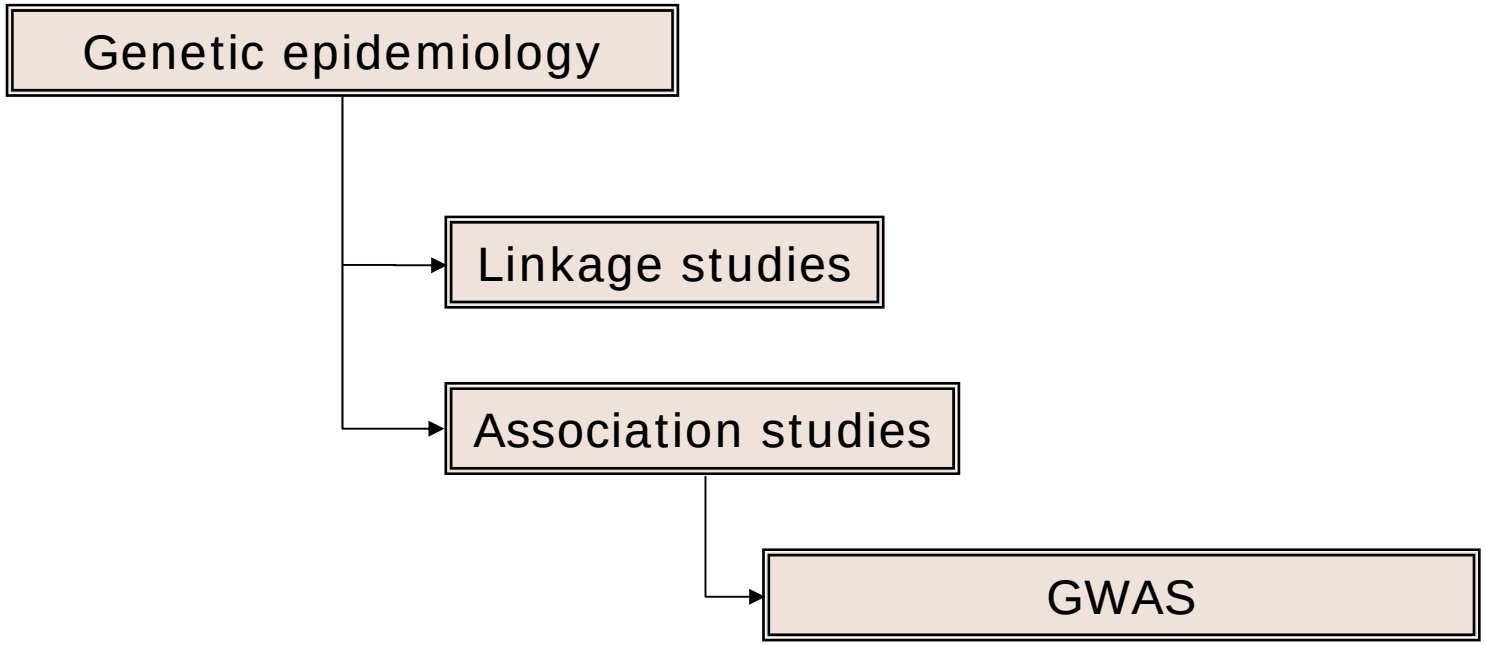
I Overview

Terminology

- Genes, Chromosome, markers
- Alleles, genotypes, haplotypes
- Phenotypes, mode of inheritance, penetrance
- Mendelian laws of inheritance, Hardy-Weinberg equilibrium, linkage disequilibrium
- Association tests for single or multiple SNPs
- Population stratification
- Multiple testing
- Gene-environment interaction (GEI)

Topics

- Organization



- The landscape
- Study design

Genetic epidemiology

- It is the study of the role of genetic factors in determining health and disease in families and in populations, and the interplay of such genetic factors with environmental factors, or “a science which deals with the aetiology, distribution, and control of diseases in groups of relatives and with inherited causes of disease in populations” (http://en.wikipedia.org).
- It customarily includes study of familial aggregation, segregation, linkage and association. It is closely associated with the development of statistical methods for human genetics which deals with these four questions. The last two questions can only be answered if appropriate genetic markers available (Elston & Ann Spence. *Stat Med* 2006;25:3049-80).

Linkage studies

- It is the study of cosegregation between genetic markers and putative disease loci, and has been very successful in localizing rare, Mendelian disorders but since has difficulty for traits which do not strictly follow Mendelian mode of inheritance, considerable linkage heterogeneity and it has limited resolution.
- It typically involves parametric (model-based) and nonparametric (model-free) methods, the latter most commonly refers to allele-sharing methods.
- The underlying concepts are nevertheless very important. It can still be useful in providing candidates for fine-mapping and association studies.
- With availability of whole genome data, it is possible to infer relationship or correlation between any individuals in a population.

Association studies

- They focus on association between particular allele and trait; it is only feasible with availability of dense markers.
- It has traditionally applied to both relatives in families and population sample. For the latter there has been serious concern over spurious association due to difference in allele frequencies between hidden sub-populations in a sample.
- A range of considerations has been made (Balding. *Nat Rev Genet* 2006;7:781-91) but the availability of whole genome data refreshes our understanding and perspectives.

GWAS

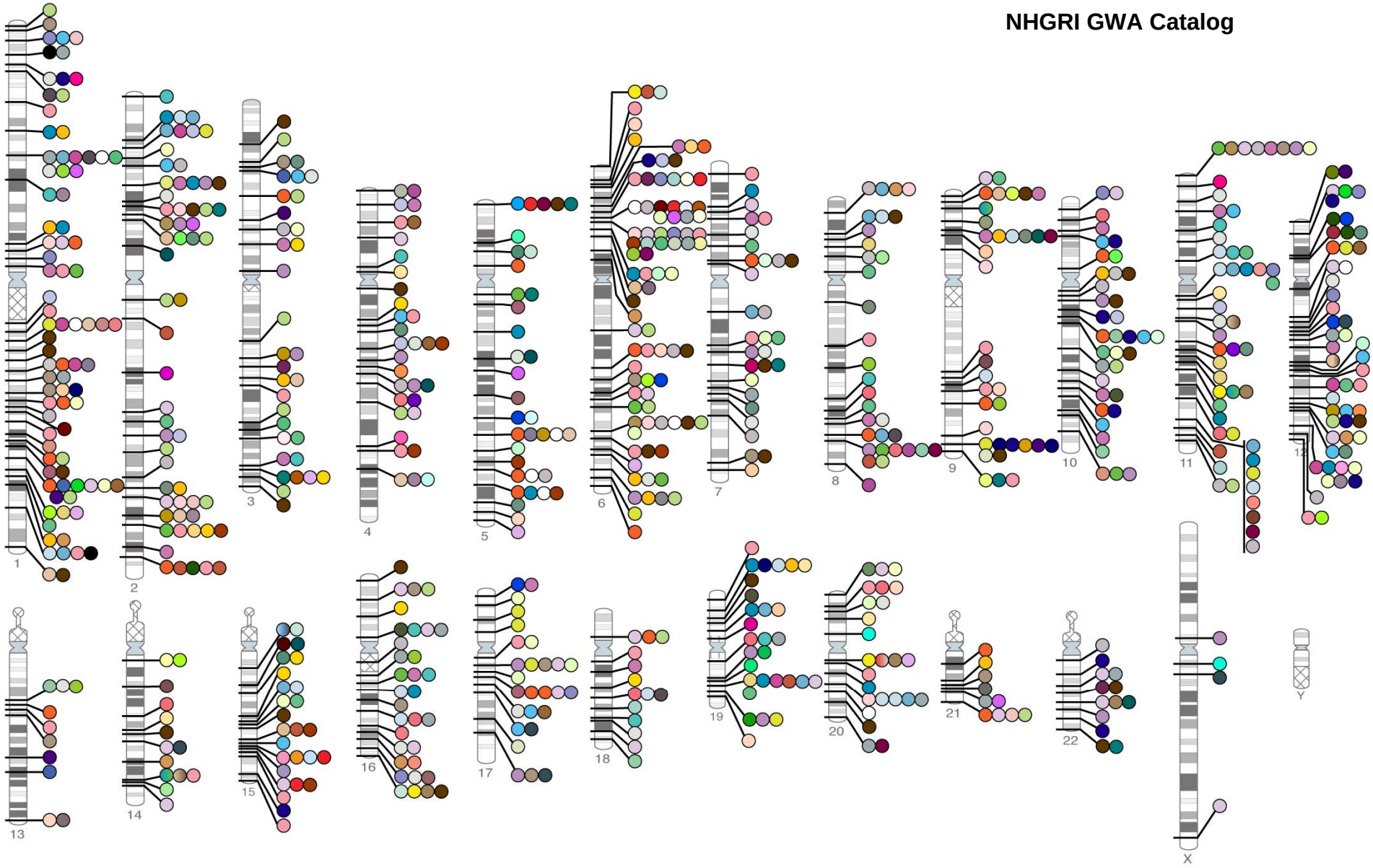
- Any study of genetic variation across the entire human genome designed to identify genetic association with observable traits or the presence or absence of a disease, usually referring to studies with genetic marker density of 100,000 or more to represent a large proportion of variation in the human genome (Pearson & Manolio. *JAMA* 2008;299:1335-44), or simply … look for associations between DNA sequence variants and phenotypes of interest (Donnelly. *Nature 2008;* 456:728-31).
- It is associated with the common disease common variant hypothesis (CD-CV). Common polymorphisms (MAF>1%) might contribute to susceptibility to common diseases, so that GWAS of common variants might be used to map loci contributing to common diseases. It therefore helps to catalog millions of common variants in the human population, massive genotypes to large number of individuals, and appropriate analytical framework (Altshuler et al. *Science* 2008; 322:881-888).

The landscape

- A catalog of published GWASs is maintained by Office of Population Genomics at the National Human Genome Research Institute (NHGRI) and available from **http://www.genome.gov/GWAStudies**
- As of 3/2010, there were 779 published genome-wide associations at $p \leq 5x10\text{-}8$ for 148 traits. As of 06/2010, the table included 587 publications. For instance, for body mass index, it includes the major publications from GWASs with 100000 SNPs, namely, Thorleifsson et al. *Nat Genet* 2009;41:18-24, Willer et al. *Nat Genet* 2009; 41:25-34; Loos et al. *Nat Genet* 2008;40:768-75, Fox et al. *BMC Med Genet* 2007;8:S18, Frayling et al. *Science* 2007;316:889-94.
- Furthermore, there were Benzinou et al. *Nat Genet* 2008;40:943-5, Meyre et al. *Nat Genet* 2009;41:157-9.

Published Genome-Wide Associations through 3/2010, 779 published GWA at $p \leq 5 \times 10^{-8}$ for 148 traits

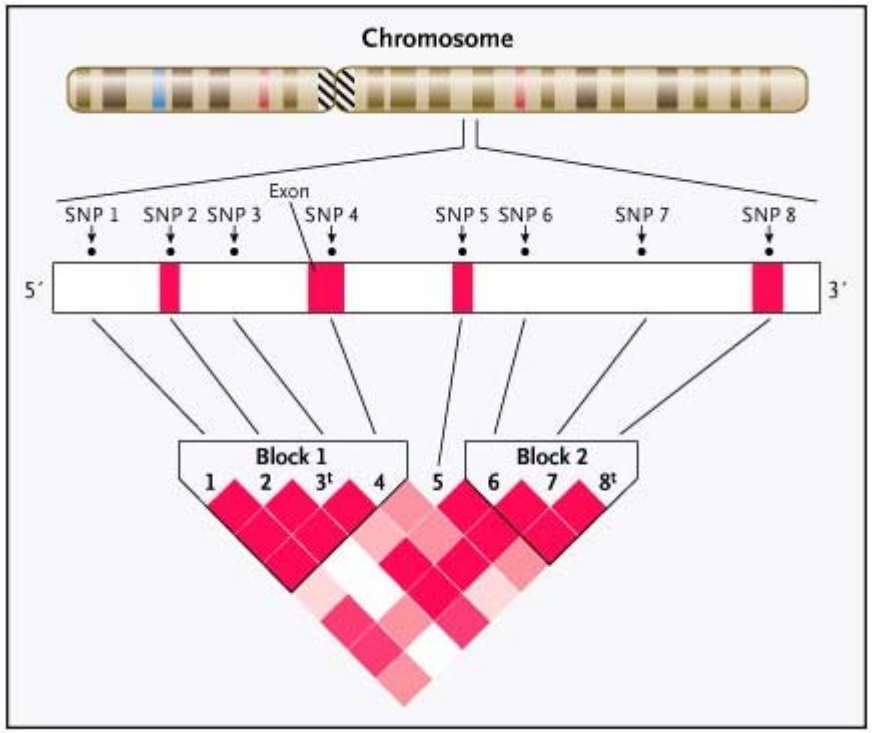
NHGRI GWA Catalog




Acute lymphoblastic leukemia
Adhesion molecules
Adiponectin levels
Age-related macular degeneration
AIDS progression
Alcohol dependence
Alzheimer disease
Amyotrophic lateral sclerosis
Angiotensin-converting enzyme activity
Ankylosing spondylitis
Arterial stiffness
Asthma
Atherosclerosis in HIV
Atrial fibrillation
Attention deficit hyperactivity disorder
Autism
Basal cell cancer
Bipolar disorder
Bilirubin
Bladder cancer
Blond or brown hair
Blood pressure
Blue or green eyes
BMI, waist circumference
Bone density
Breast cancer
C-reactive protein
Cardiac structure/function
Carnitine levels
Carotenoid/tocopherol levels
Celiac disease
Chronic lymphocytic leukemia
Cleft lip/palate
Cognitive function
Colorectal cancer
Coronary disease
Creutzfeldt-Jakob disease
Crohn's disease
Cutaneous nevi
Dermatitis
Drug-induced liver injury
Eosinophil count
Eosinophilic esophagitis
Erythrocyte parameters
Esophageal cancer
Essential tremor
Exfoliation glaucoma
F cell distribution
Fibrinogen levels
Folate pathway vitamins
Freckles and burning
Gallstones
Glioma
Glycemic traits
Hair color
Hair morphology
HDL cholesterol
Heart rate
Height
Hemostasis parameters
Hepatitis
Hirschsprung's disease
HIV-1 control
Homocysteine levels
Idiopathic pulmonary fibrosis
IgE levels
Inflammatory bowel disease
Intracranial aneurysm
Iris color
Iron status markers
Ischemic stroke
Juvenile idiopathic arthritis
Kidney stones
LDL cholesterol
Leprosy
Leptin receptor levels
Liver enzymes
LP (a) levels
Lung cancer
Major mood disorders
Malaria
Male pattern baldness
Matrix metalloproteinase levels
MCP-1
Melanoma
Menarche & menopause
Multiple sclerosis
Myeloproliferative neoplasms
Narcolepsy
Nasopharyngeal cancer
Neuroblastoma
Nicotine dependence
Obesity
Open personality
Osteoarthritis
Osteoporosis
Otosclerosis
Other metabolic traits
Ovarian cancer
Pain
Pancreatic cancer
Panic disorder
Parkinson's disease
Periodontitis
Peripheral arterial disease
Phosphatidylcholine levels
Platelet count
Primary biliary cirrhosis
PR interval
Prostate cancer
Protein levels
Psoriasis
Pulmonary funct. COPD
QRS interval
QT interval
Quantitative traits
Recombination rate
Red vs.non-red hair
Renal function
Response to antipsychotic therapy
Response to hepatitis C treatment
Response to statin therapy
Restless legs syndrome
Rheumatoid arthritis
Schizophrenia
Serum metabolites
Skin pigmentation
Speech perception
Sphingolipid levels
Statin-induced myopathy
Stroke
Systemic lupus erythematosus
Telomere length
Testicular germ cell tumor
Thyroid cancer
Tooth development
Total cholesterol
Triglycerides
Type 1 diabetes
Type 2 diabetes
Ulcerative colitis
Urate
Venous thromboembolism
Vitamin B12 levels
Warfarin dose
Weight
White cell count
YKL-40 levels

Context

- The population under study must be characterized to allow the selection of patients likely to share a genetic cause of disease
- Thousands of cases and controls may be needed if a study is to have sufficient statistical power to identify the alleles of interest
- … it creates bioinformatics challenges and raises questions about how to identify true positive signals

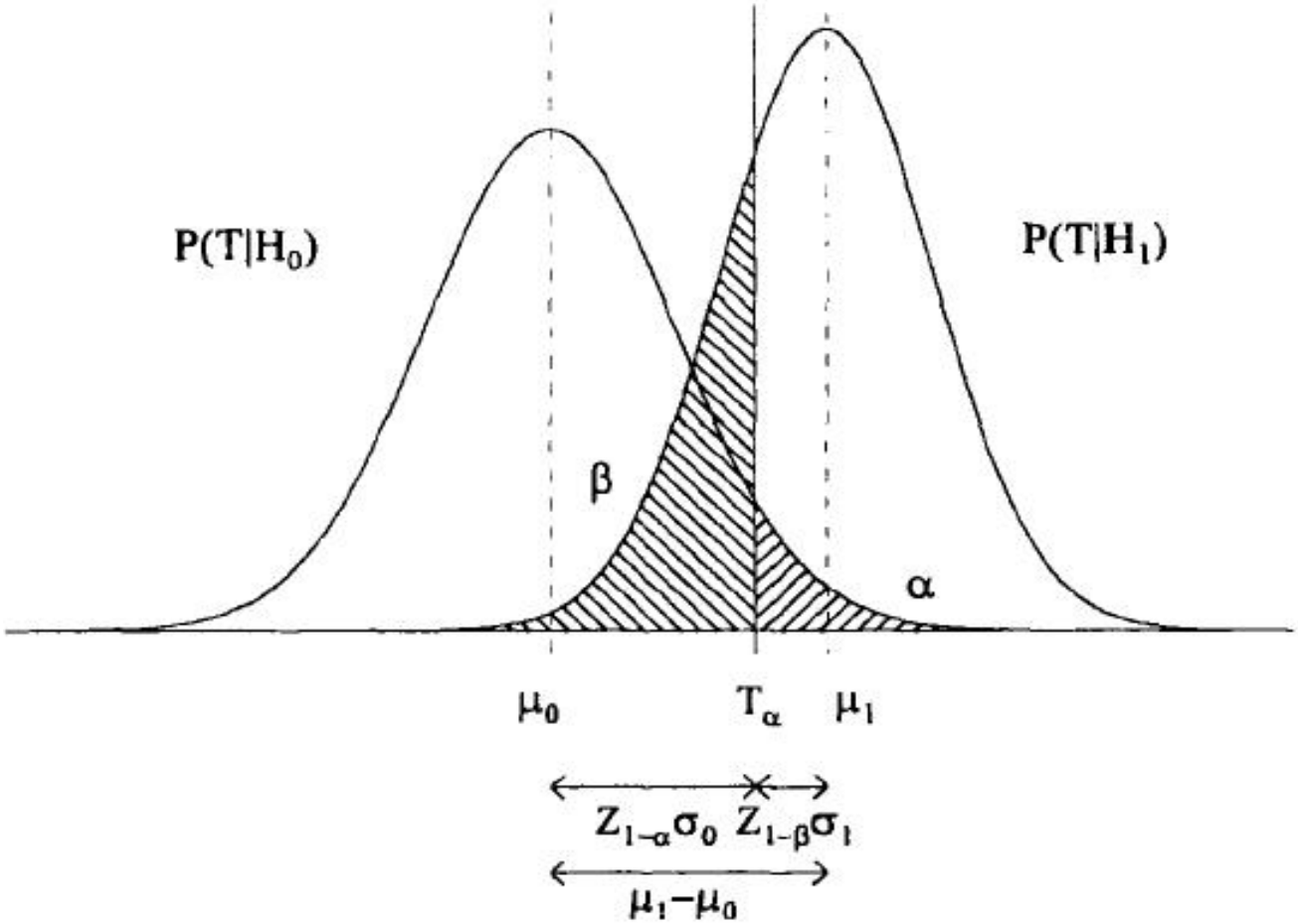


Christensen & Murray. *New Eng J Med* 2007;356:1094-7

International collaborative projects

- The HapMap project (http://hapmap.ncbi.nlm.nih.gov/) was a study of 270 people from the Yoruba in Nigeria (30 trios), Japanese (45 unrelated individuals), Han Chinese (45 unrelated individuals) and CEPH (30 trios).
- The 1000 genome project (http://www.1000genomes.org) aims to sequence at least one thousand anonymous participants. It still undergoes revision.
- The database of genotypes and phenotypes (dbGaP) (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap) was developed to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype.
- The genetic analysis workshops (GAWs) (http://www.gaworkshop.org/) are a collaborative effort among genetic epidemiologists to evaluate and compare statistical genetic methods. For each GAW, topics are chosen that are relevant to current analytical problems through simulated or real data.

A conceptual picture based on a test of H_0: $\mu=\mu_0$ vs H_1: $\mu=\mu_1>\mu_0$ from a normal distribution



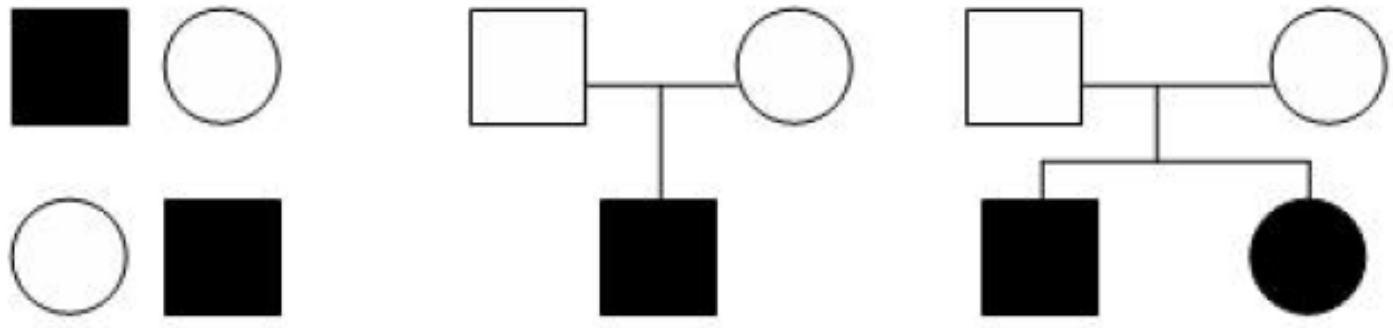
Sample size calculation for normal distribution

Let $T \sim N(\mu_1, \sigma_1^2)$, we have the following steps,

- $Z = \frac{T-\mu_0}{\sigma_0} \sim N(\frac{\mu_1-\mu_0}{\sigma_0}, \frac{\sigma_1^2}{\sigma_0^2})$.
- $\beta = P(Z < Z_{1-\alpha}|\mu_1, \sigma_1^2) = \Phi\left(\frac{Z_{1-\alpha} - \frac{\mu_1-\mu_0}{\sigma_0}}{\frac{\sigma_1}{\sigma_0}}\right)$ and $Z_\beta = \frac{Z_{1-\alpha}\sigma_0 - (\mu_1-\mu_0)}{\sigma_1}$.
- Since $Z_\beta = -Z_{1-\beta}$ and we are interested in $1-\beta$, $Z_{1-\beta} = \frac{(\mu_1-\mu_0) - Z_{1-\alpha}\sigma_0}{\sigma_1}$, $|\mu_1 - \mu_0| = Z_{1-\alpha}\sigma_0 + Z_{1-\beta}\sigma_1$.
- As $\sigma_i \equiv \sigma_i/N$, $i = 1, 2$. $\sqrt{N}|\mu_1 - \mu_0| = Z_{1-\alpha}\sigma_0 + Z_{1-\beta}\sigma_1$.

$$N = \left(\frac{Z_{1-\alpha}\sigma_0 + Z_{1-\beta}\sigma_1}{\mu_1 - \mu_0}\right)^2$$

Study designs



- Three common genetic association designs involving unrelated individuals (left), nuclear families with affected singletons (middle) and affected sib-pairs (right). Males and females are denoted by squares and circles with affected individuals filled with black colors and unaffected individuals being empty
- Risch & Merikangas. *Science* 1996;273:1516-7, Zhao. *J Stat Soft* 2007;23(8):1-18

Sample sizes required for association detection using population data

		K			
γ	p	1%	5%	10%	20%
4.0	0.01	46638	8951	4240	1885
	0.10	8173	1569	743	331
	0.50	10881	2089	990	440
	0.80	31444	6035	2859	1271
2.0	0.01	403594	77458	36691	16307
	0.10	52660	10107	4788	2128
	0.50	35252	6766	3205	1425
	0.80	79317	15223	7211	3205
1.5	0.01	1598430	306770	145312	64583
	0.10	191926	36835	17448	7755
	0.50	97922	18793	8902	3957
	0.80	191926	36835	17448	7755

Power of linkage versus association

		Linkage			Association					
γ	p	Y	N_L	P_A	H_1	N_{tdt}	H_2	$N_{asp/tdt}$	λ_o	λ_s
4.00	0.01	0.520	6400	0.800	0.048	1098	0.112	235	1.08	1.09
	0.10	0.597	277	0.800	0.346	151	0.537	48	1.48	1.54
	0.50	0.576	445	0.800	0.500	104	0.424	62	1.36	1.39
	0.80	0.529	3023	0.800	0.235	223	0.163	162	1.12	1.13
2.00	0.01	0.502	445839	0.667	0.029	5824	0.043	1970	1.01	1.01
	0.10	0.518	8085	0.667	0.245	696	0.323	265	1.07	1.08
	0.50	0.526	3752	0.667	0.500	340	0.474	180	1.11	1.11
	0.80	0.512	17904	0.667	0.267	640	0.217	394	1.05	1.05
1.50	0.01	0.501	6942837	0.600	0.025	19321	0.031	7777	1.00	1.00
	0.10	0.505	101898	0.600	0.214	2219	0.253	941	1.02	1.02
	0.50	0.510	27041	0.600	0.500	950	0.490	485	1.04	1.04
	0.80	0.505	101898	0.600	0.286	1663	0.253	941	1.02	1.02

Power calculation under matched design

- We can use conditional logistic regression model

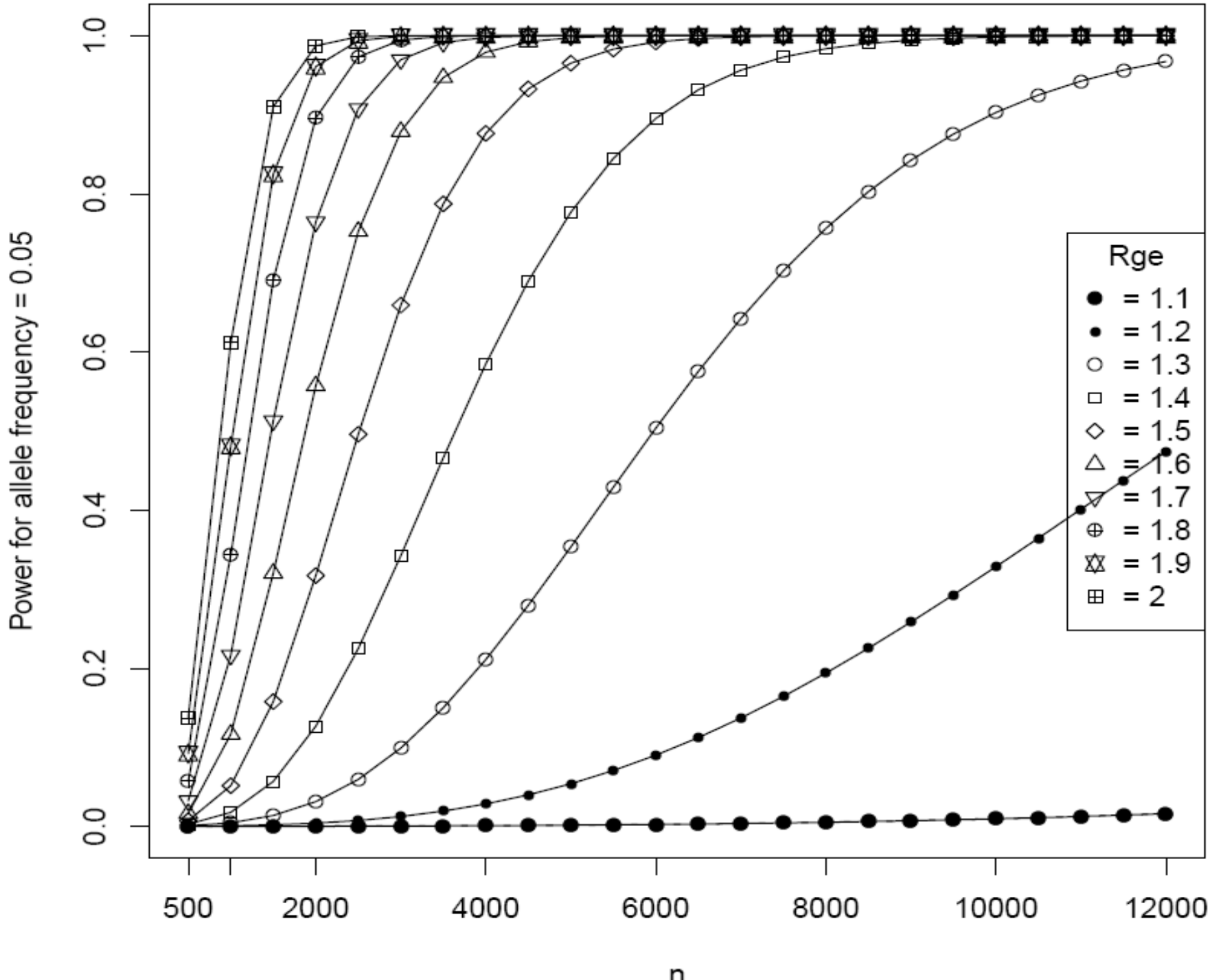
$$L(\beta_g, \beta_e, \beta_{ge}) = \prod_{i=1}^{N} \frac{e^{\beta_g G_{ij} + \beta_e E_{ij} + \beta_{ge} G_{ij} E_{ij}}}{\sum_{j \in M(i)} e^{\beta_g G_{ij} + \beta_e E_{ij} + \beta_{ge} G_{ij} E_{ij}}}$$

  where $M(i)$ includes all subjects in matched set i.

- Power/sample size calculation can proceed with contrasting $l^1 = \ln[L(\beta_g, \beta_e, \beta_{ge})]$, $l^0 = \ln[L(\beta_g, \beta_e)]$ with $\Lambda = 2(\hat{l}^1 - \hat{l}^0)$ and $N\Lambda$ being the non-centrality parameter of chi-squared distribtion under the alternative hypothesis.
- The required sample size is obtained via equating the noncentrality parameter to theoretical values under a given significant level and power (the previous conceptual picture still applies).

GEI of type-2 diabetes

- Legends in the project manual were perhaps confusing so it is worthwhile to re-present here.
  - Matched case-control study
  - Type I error rate (α) = 0.00001 (two-sided)
  - Continuous environmental factors with standard deviation 1, and interaction odds ratio (*Rge*) = 1.2 ~ 4
  - K = 0.05 (done for 0.1 ~ 0.15)
  - Sample size (N) = 500 ~ 12,000
  - Additive model
  - Allele frequency (p) = 0.05, 0.1, 0.2, 0.3
- We supplied these to Quanto 1.0 (http://hydra.usc.edu/gxe, now available on the Epidemiology Unit machines)
- Gauderman WJ. *Stat Med* 21:35-50, 2002


Power for allele frequency = 0.05
0.0
0.2
0.4
0.6
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1.0
500
2000
4000
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8000
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12000
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Rge
= 1.1
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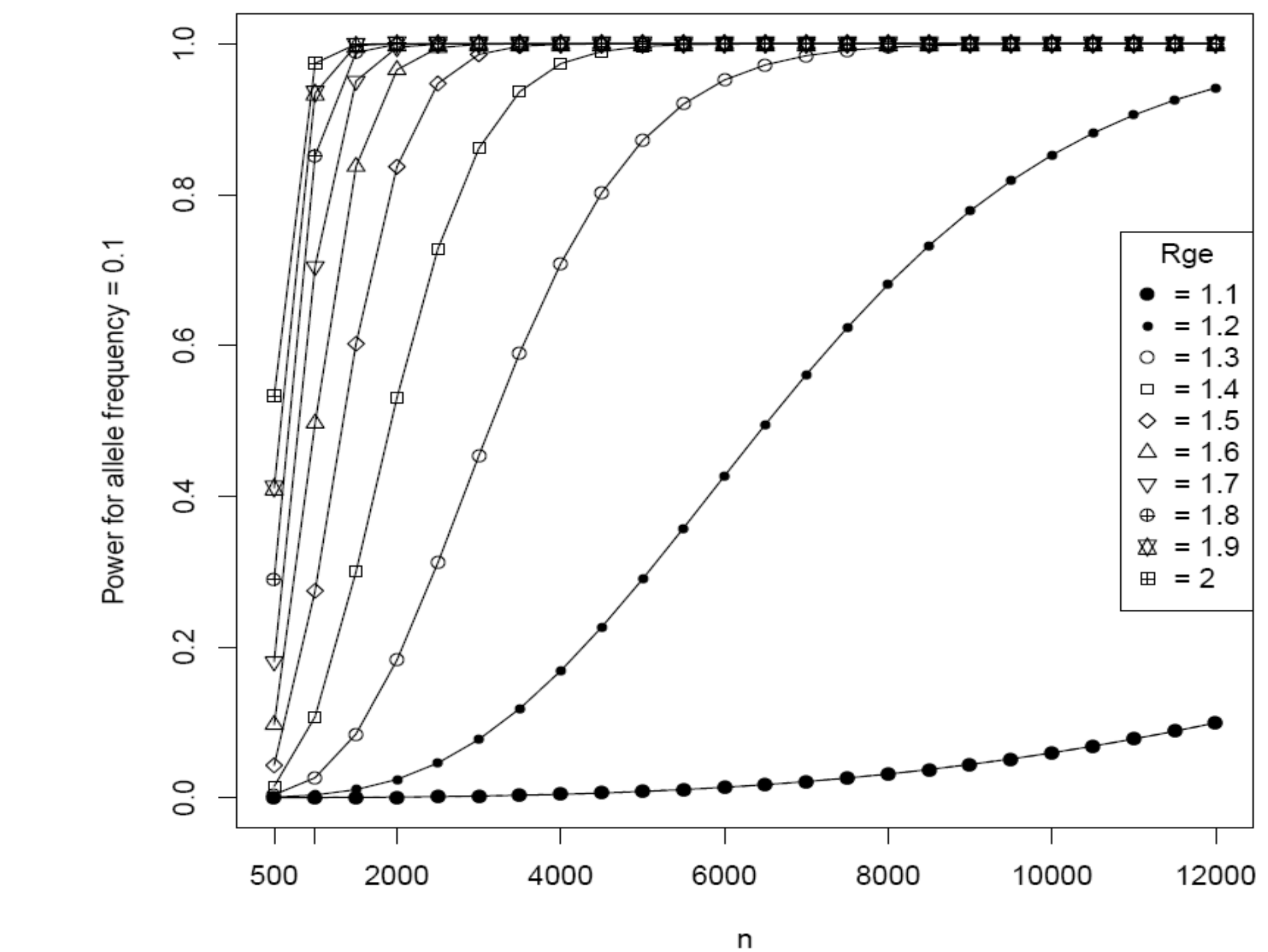
Power for allele frequency = 0.1
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Rge
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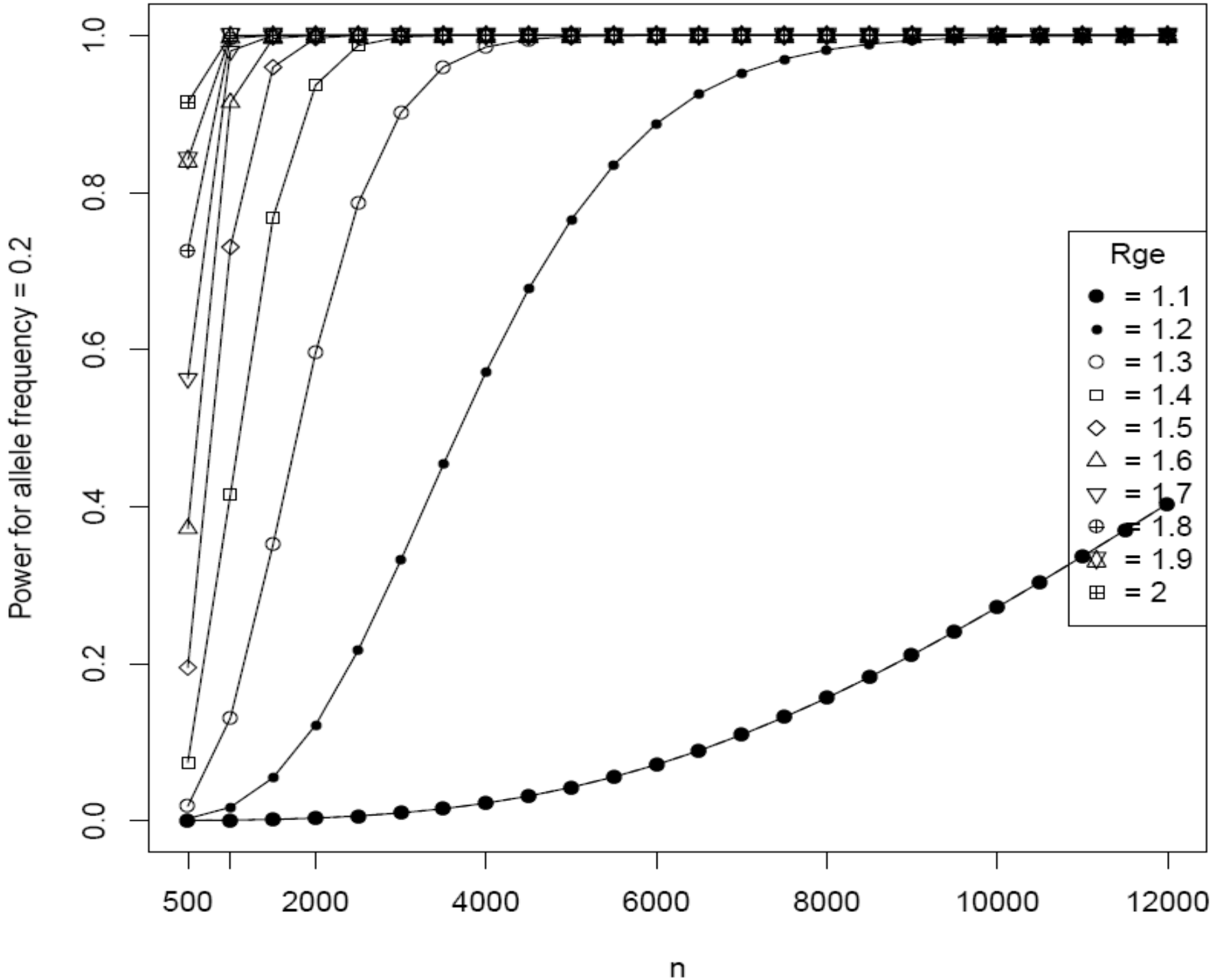
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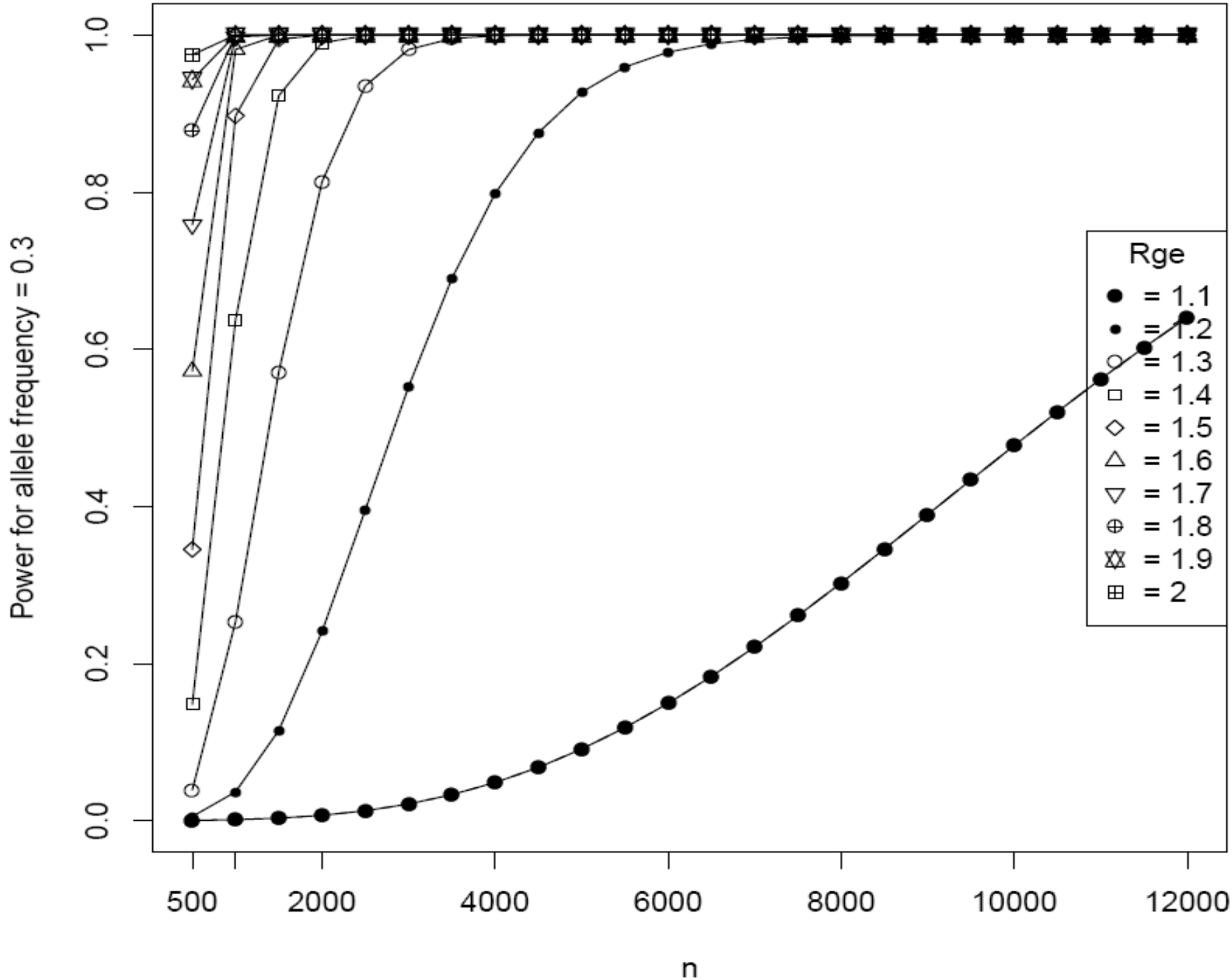
Power for allele frequency = 0.3
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Rge
= 1.1
= 1.2
= 1.3
= 1.4
= 1.5
= 1.6
= 1.7
= 1.8
= 1.9
= 2

One more example of EDNAR application

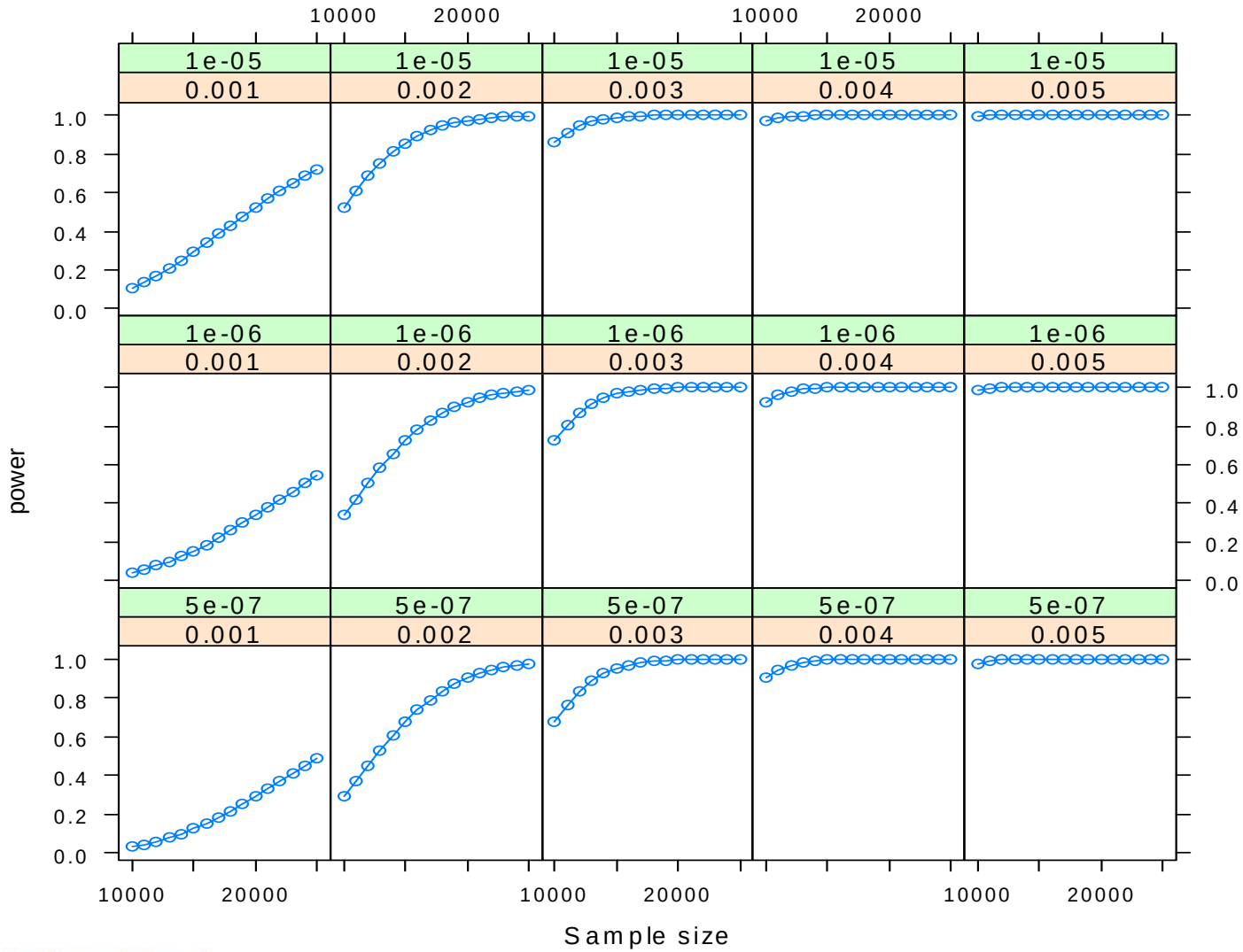
- The calculation is as a linear function of proportion of variance explained, significant level and sample size.

```
proc power;
    ods output output=op;
    multreg
        model = fixed
        alpha = 0.00001 0.000001 0.0000005
        nfullpred = 1
        ntestpred = 1
        rsqfull = 0.001 to 0.005 by 0.001
        rsqdiff = 0.001 to 0.005 by 0.001
        ntotal = 10000 to 25000 by 1000
        power = .;
run;
```

Power by %variance explained (R^2)

	R^2				
Sample size	0.1	0.2	0.3	0.4	0.5
$\alpha = 10^{-5}$					
10000	0.10	0.52	0.86	0.97	1
15000	0.29	0.86	0.99	1.00	1
20000	0.52	0.97	1.00	1.00	1
25000	0.72	1.00	1.00	1.00	1
$\alpha = 5x10^{-7}$					
10000	0.031	0.29	0.67	0.9	0.98
15000	0.124	0.67	0.95	1.0	1.00
20000	0.290	0.90	1.00	1.0	1.00
25000	0.489	0.98	1.00	1.0	1.00

Power by α =1e-5,1e-6,5e-7



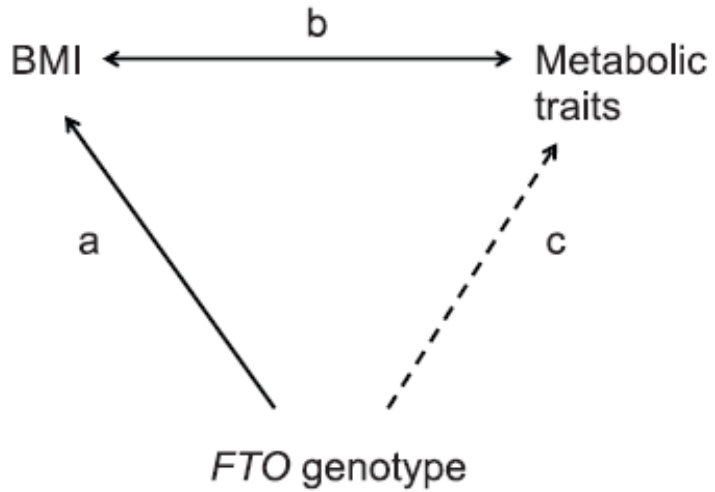
Two-stage design on main effect

- The goal is to reduce cost without compromising efficiency. Given our study sample and SNPs of interest are defined, a staged design furnishes collection of all information in several steps.
- In the simplest and well-studied two-staged design of genetic case-controls studies, a proportion of individuals is genotyped at all of the SNPs and a proportion of the most significant ones is selected and to be carried over as replication study at the second stage.
- Skol et al. *Nat Genet* 2006, 38(2):209-13 (check the associate website for a program called CaTS).
- It is implemented in the function tscc within R/gap.

FTO-BMI-T2D Mendelian randomisation

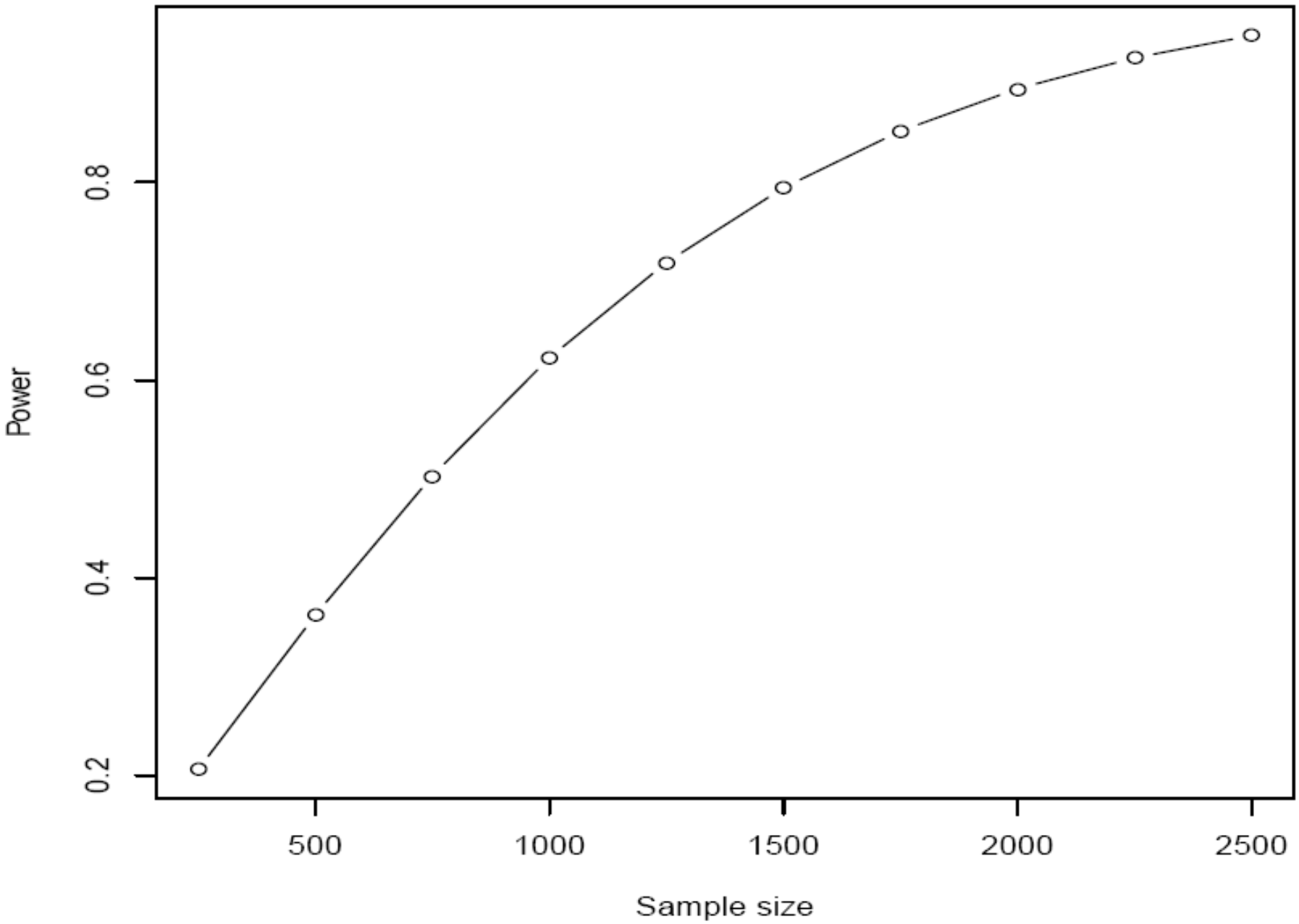
- *FTO*-T2D association is gone once BMI is included in the model. This has been used in the so-called Mendelian randomisation study disentangling the causal association of BMI-T2D (Freathy *et al*. *Diabetes* 2008, 57:1419-26).

There is association between *FTO* and BMI (a). There is epidemiological association between BMI and metabolic traits (b). The association between *FTO* genotype and metabolic traits is mediated by BMI (c=axb).



Power calculation

- We can of course perform simulations to obtain power estimate but it would be somewhat involved.
- Instead, we calculate standard error of *FTO*-BMI-T2D can be calculated which can form the basis of power calculation (Kline RB. Principles and Practice of Structural Equation Modeling, 2nd Edition, The Guilford Press 2005).
- We implement this in *ab* function in R/gap.
- We have for EPIC-Norfolk 25,000, SNP-BMI regression coefficient (SE) of 0.15 (0.01), and BMI-T2D log(1.19) (0.01). We consider α=0.05.
- Criticism arised from this posthoc power calculation could be alleviated when we allow for a range of sample sizes to be considered in the next slide.


Power
0.2
0.4
0.6
0.8
500
1000
1500
2000
2500
Sample size

Two-stage GEI

- A case-only design is used as the first stage.
- This is to be followed by a second stage involving both cases and controls.

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Murcray et al. *Am J Epidemiol* 2008; 169:219-26

Li & Conti. *Am J Epidemiol* 2008; 169:497-504

Thomas D. *Nat Rev Genet* 2010 (Epub)

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A summary

- We have restricted our focus and leave out a lot of details to cover a rapid moving field with limited time.
- It seems that the practice of study designs and data analysis cannot be changed in a short run, but we have already seen steady increase in use of R.

Case study: GWAS of obesity-related traits

- Background
- Study design
- Statistical analysis
- On-going research

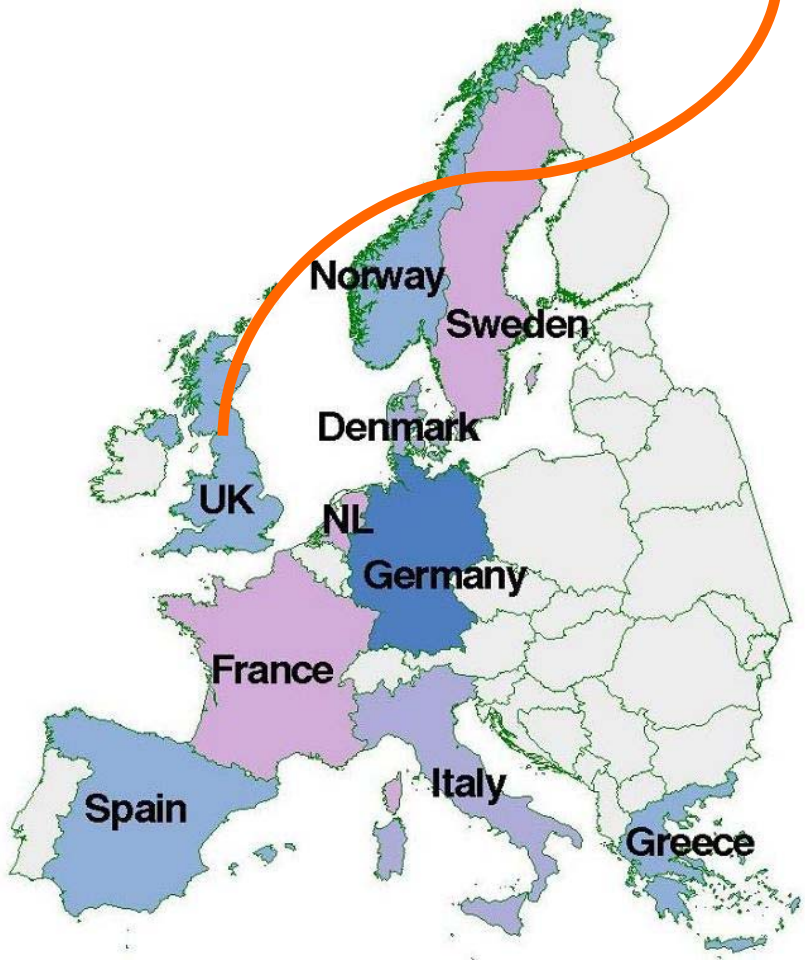
EPIC study

The European Prospective Investigation into Cancer and Nutrition (EPIC) is coordinated by Dr Elio Riboli, Head of the Division of Epidemiology, Public Health and Primary Care at the Imperial College London.

EPIC was designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors and the incidence of cancer and other chronic diseases. EPIC is the largest study of diet and health ever undertaken, having recruited over half a million (520,000) people in ten European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom.

EPIC-Norfolk study

EPIC-Norfolk participants are men and women (based on over 30,000 people) who were aged between 45 and 74 when they joined the study, who lived in Norwich and the surrounding towns and rural areas. They have been contributing information about their diet, lifestyle and health through questionnaires, and through health checks carried out by EPIC nurses.

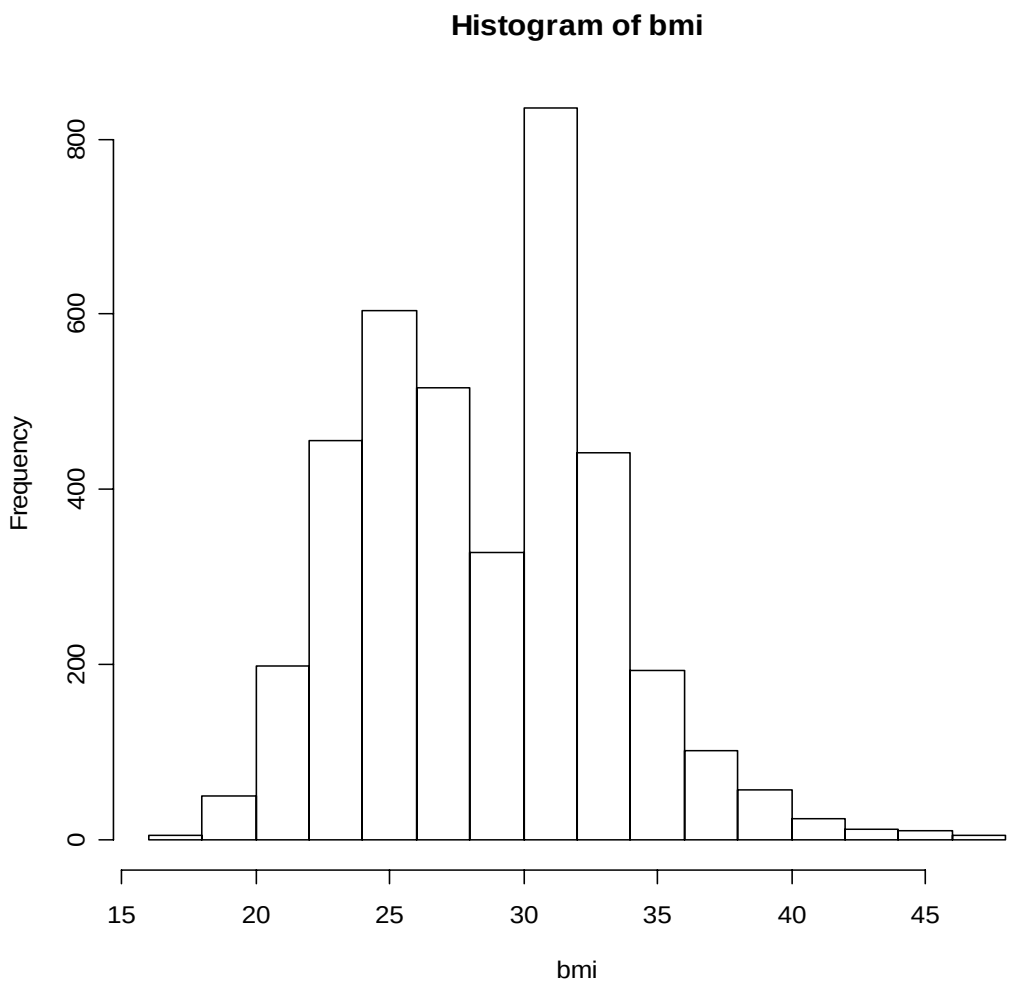


Case-cohort design for EPIC-Norfolk study

- It originally followed case-control design (e.g., WTCCC with seven cases and common controls) with 3425 cases and 3400 controls.
  - It is potentially more powerful.
  - Controls are selected.
- It has then been changed into case-cohort design, in which cases are defined to be individuals whose BMI above 30 and controls are a random sample (subcohort) of the EPIC-Norfolk cohort which includes obese individuals.
  - The subcohort is representative of the whole population and allows for a range of traits to be examined.
  - The analysis is potentially more involved but established.

Case-cohort design

- The distribution of body mass index (BMI) is the case-cohort design of the EPIC-Norfolk study of obesity is a combination of the sub-cohort sample and case sample which is truncated from the whole cohort at BMI=30
- Zhao. *J Stat Soft* 2007;23(8):1-18



Power/sample size

- It started with assessment of how the power is compromised relative to the original case-control design.
- This was followed by power/sample size calculation using methods established by Cai and Zeng (2004) as implemented in an R function, noting a number of assumptions.
- More practically, it was also envisaged that a proper representative sample of a total of 25,000 individuals would be 10%; the subcohort is then approximately 2,500.
- The total sample was split between two stages.

GeneChips

- Affymetrix 500K
  - Data were available for 3850 individuals
- Illumina 317K
  - It came at a later time。
  - Data quality appears to be poor?
- The focus has therefore been Affy500K, but with a possible comeback.

Analysis

- An incremental approach was adopted since the storage and computing power were somewhat uncertain.
- This was predated with controls from the breast cancer study, involving about 400 individuals with Perlegen 250K GeneChips.
- QC including call rates and HWE was feasible with SAS/Genetics (~30GB) which provides a good estimate of the storage for all individuals (~380GB).
- The Linux platform seemed favourable.

EPIC400 analysis

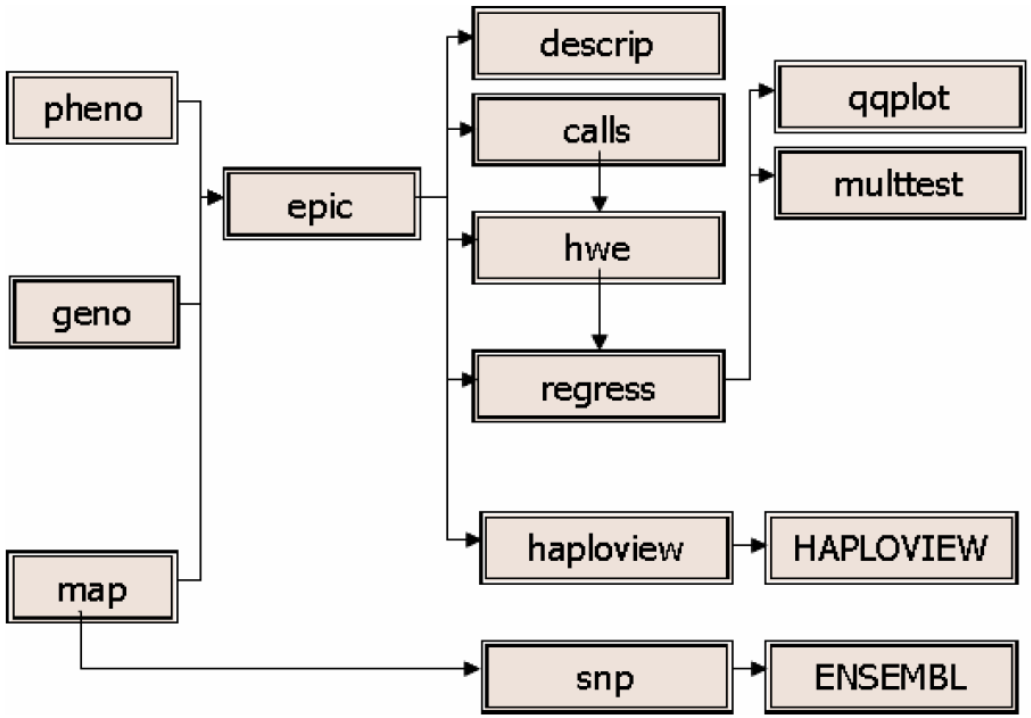


Fig. 1. A flowchart of the EPIC 400 analysis, with modules in brackets. Genotypes (geno) and phenotypes (pheno) are merged (epic) for descriptive statistics (descrip) call rates (calls), HWE (hwe), regression (regress) with adjustment for multiple testing (multtest) and comparison with theoretical distribution (qqplot). The raw data together with map information (map) can also be reformatted (haploview) into HAPLOVIEW input files so that specific region in the genome can be visualized, with annotation information from ENSEMBL according to SNPs (snp).

The analysis for GWAS

- QC including visualisation of clustering, outliers, was largely done by colleagues at Sanger (as for WTCCC)
- The overall strategy was data partition, i.e., by chromosome and further by region (30) in each chromosome, largely on a long, skinny data format
- A major advantage is that the analysis can be resumed whenever the system experiences problems
- We stuck to SAS to allow for reliability and flexibility with or without SAS/Genetics, for BMI/obesity as continuous and binary outcomes are readily tackled with REG/LOGISTIC procedures – most outputs are available from the output delivery system (ODS)
- The picture was eventually changed with a revised coding algorithm and the use of imputed data

Additional analysis

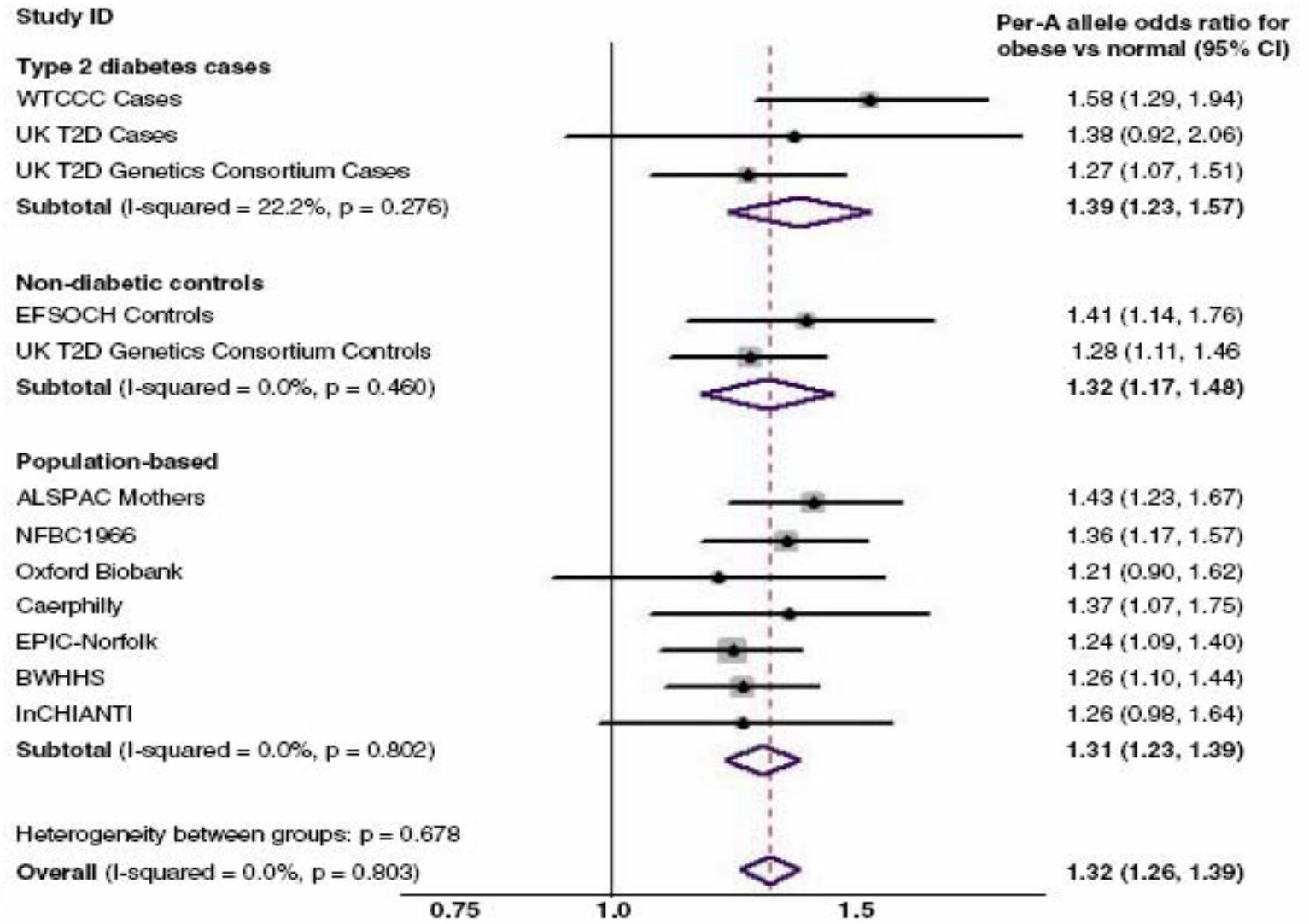
- Population stratification via EIGENSTRAT
  - SAS is very handy since a single put statement is sufficient to generate the output.
- Collaborative (e.g. height) and consortium work (GIANT)
  - On the UK side, this is mainly involved with IMPUTE/SNPTEST, with inputs on strand, standard error, quantitative traits, outputs.
  - This facilitates meta-analysis considerably.

The first report

A Common Variant in the *FTO* Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity

Timothy M. Frayling,[1,2]* Nicholas J. Timpson,[3,4]* Michael N. Weedon,[1,2]* Eleftheria Zeggini,[3,5]* Rachel M. Freathy,[1,2] Cecilia M. Lindgren,[3,5] John R. B. Perry,[1,2] Katherine S. Elliott,[3] Hana Lango,[1,2] Nigel W. Rayner,[3,5] Beverley Shields,[2] Lorna W. Harries,[2] Jeffrey C. Barrett,[3] Sian Ellard,[2,6] Christopher J. Groves,[5] Bridget Knight,[2] Ann-Marie Patch,[2,6] Andrew R. Ness,[7] Shah Ebrahim,[8] Debbie A. Lawlor,[9] Susan M. Ring,[9] Yoav Ben-Shlomo,[9] Marjo-Riitta Jarvelin,[10,11] Ulla Sovio,[10,11] Amanda J. Bennett,[5] David Melzer,[1,12] Luigi Ferrucci,[13] Ruth J. F. Loos,[14] Inês Barroso,[15] Nicholas J. Wareham,[14] Fredrik Karpe,[5] Katharine R. Owen,[5] Lon R. Cardon,[3] Mark Walker,[16] Graham A. Hitman,[17] Colin N. A. Palmer,[18] Alex S. F. Doney,[19] Andrew D. Morris,[19] George Davey Smith,[4] The Wellcome Trust Case Control Consortium,† Andrew T. Hattersley,[1,2]‡§ Mark I. McCarthy[3,5]‡

Meta-analysis for odds of obesity



LDL

LDL-cholesterol concentrations: a genome-wide association study

Manjinder S Sandhu, Dawn M Waterworth*, Sally L Debenham*, Eleanor Wheeler, Konstantinos Papadakis, Jing Hua Zhao, Kijoung Song, Xin Yuan, Toby Johnson, Sofie Ashford, Michael Inouye, Robert Luben, Matthew Sims, David Hadley, Wendy McArdle, Philip Barter, Y Antero Kesäniemi, Robert W Mahley, Ruth McPherson, Scott M Grundy, Wellcome Trust Case Control Consortium†, Sheila A Bingham, Kay-Tee Khaw, Ruth J F Loos, Gérard Waeber, Inês Barroso, David P Strachan, Panagiotis Deloukas, Peter Vollenweider, Nicholas J Wareham, Vincent Mooser*

Height

Genome-wide association analysis identifies 20 loci that influence adult height

Michael N Weedon[1,2,23], Hana Lango[1,2,23], Cecilia M Lindgren[3,4], Chris Wallace[5], David M Evans[6], Massimo Mangino[7], Rachel M Freathy[1,2], John R B Perry[1,2], Suzanne Stevens[7], Alistair S Hall[8], Nilesh J Samani[7], Beverly Shields[2], Inga Prokopenko[3,4], Martin Farrall[9], Anna Dominiczak[10], Diabetes Genetics Initiative[21], The Wellcome Trust Case Control Consortium[21], Toby Johnson[11–13], Sven Bergmann[11,12], Jacques S Beckmann[11,14], Peter Vollenweider[15], Dawn M Waterworth[16], Vincent Mooser[16], Colin N A Palmer[17], Andrew D Morris[18], Willem H Ouwehand[19,20], Cambridge GEM Consortium[22], Mark Caulfield[5], Patricia B Munroe[5], Andrew T Hattersley[1,2], Mark I McCarthy[3,4] & Timothy M Frayling[1,2]

Adult height is a model polygenic trait, but there has been limited success in identifying the genes underlying its normal variation. To identify genetic variants influencing adult human height, we used genome-wide association data from 13,665 individuals and genotyped 39 variants in an additional 16,482 samples. We identified 20 variants associated with adult height ($P < 5 \times 10^{-7}$, with 10 reaching $P < 1 \times 10^{-10}$). Combined, the 20 SNPs explain ~3% of height variation, with a ~5 cm difference between the 6.2% of people with 17 or fewer 'tall' alleles compared to the 5.5% with 27 or more 'tall' alleles. The loci we identified implicate genes in Hedgehog signaling (*IHH, HHIP, PTCH1*), extracellular matrix (*EFEMP1, ADAMTSL3, ACAN*) and cancer (*CDK6, HMGA2, DLEU7*) pathways, and provide new insights into human growth and developmental processes. Finally, our results provide insights into the genetic architecture of a classic quantitative trait.

BMI/obesity

Common variants near *MC4R* are associated with fat mass, weight and risk of obesity

Ruth J F Loos[*,1,2,73], Cecilia M Lindgren[3,4,73], Shengxu Li[1,2,73], Eleanor Wheeler[5], Jing Hua Zhao[1,2], Inga Prokopenko[3,4], Michael Inouye[5], Rachel M Freathy[6,7], Antony P Attwood[5,8], Jacques S Beckmann[9,10], Sonja I Berndt[11], The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial[71], Sven Bergmann[9,12], Amanda J Bennett[3,4], Sheila A Bingham[13], Murielle Bochud[14], Morris Brown[15], Stéphane Cauchi[16], John M Connell[17], Cyrus Cooper[18], George Davey Smith[19], Ian Day[18], Christian Dina[16], Subhajyoti De[20], Emmanouil T Dermitzakis[5], Alex S F Doney[21], Katherine S Elliott[3], Paul Elliott[22,23], David M Evans[3,19], I Sadaf Farooqi[2,24], Philippe Froguel[16,25], Jilur Ghori[5], Christopher J Groves[3,4], Rhian Gwilliam[5], David Hadley[26], Alistair S Hall[27], Andrew T Hattersley[6,7], Johannes Hebebrand[28], Iris M Heid[29,30], KORA[71], Blanca Herrera[3,4], Anke Hinney[28], Sarah E Hunt[5], Marjo-Riitta Jarvelin[22,23,31], Toby Johnson[9,12,14], Jennifer D M Jolley[8], Fredrik Karpe[4], Andrew Keniry[5], Kay-Tee Khaw[32], Robert N Luben[32], Massimo Mangino[33], Jonathan Marchini[34], Wendy L McArdle[35], Ralph McGinnis[5], David Meyre[16], Patricia B Munroe[36], Andrew D Morris[21], Andrew R Ness[37], Matthew J Neville[4], Alexandra C Nica[5], Ken K Ong[1,2], Stephen O'Rahilly[2,24], Katharine R Owen[4], Colin N A Palmer[38], Konstantinos Papadakis[26], Simon Potter[5], Anneli Pouta[31,39], Lu Qi[40], Nurses' Health Study[71], Joshua C Randall[3,4], Nigel W Rayner[3,4], Susan M Ring[35], Manjinder S Sandhu[1,32], André Scherag[41], Matthew A Sims[1,2], Kijoung Song[42], Nicole Soranzo[5], Elizabeth K Speliotes[43,44], Diabetes Genetics Initiative[71], Holly E Syddall[18], Sarah A Teichmann[20], Nicholas J Timpson[3,19], Jonathan H Tobias[45], Manuela Uda[46], The SardiNIA Study[71], Carla I Ganz Vogel[28], Chris Wallace[36], Dawn M Waterworth[42], Michael N Weedon[6,7], The Wellcome Trust Case Control Consortium[72], Cristen J Willer[47], FUSION[71], Vicki L Wraight[2,24], Xin Yuan[42], Eleftheria Zeggini[3], Joel N Hirschhorn[44,48–51], David P Strachan[26], Willem H Ouwehand[8], Mark J Caulfield[36], Nilesh J Samani[33], Timothy M Frayling[6,7], Peter Vollenweider[52], Gerard Waeber[52], Vincent Mooser[42], Panos Deloukas[5], Mark I McCarthy[3,4,73], Nicholas J Wareham[1,2,73] & Inês Barroso[5,73]

Further on BMI

Six new loci associated with body mass index highlight a neuronal influence on body weight regulation

*Cristen J Willer[1,77,78], Elizabeth K Speliotes[2,3,77,78], Ruth J F Loos[4,5,77,78], Shengxu Li[4,5,77,78], Cecilia M Lindgren[6,78], Iris M Heid[7,78], Sonja I Berndt[8], Amanda L Elliott[9,10], Anne U Jackson[1], Claudia Lamina[7], Guillaume Lettre[9,11], Noha Lim[12], Helen N Lyon[3,11], Steven A McCarroll[9,10], Konstantinos Papadakis[13], Lu Qi[14,15], Joshua C Randall[6], Rosa Maria Roccasecca[16], Serena Sanna[17], Paul Scheet[18], Michael N Weedon[19], Eleanor Wheeler[16], Jing Hua Zhao[4,5], Leonie C Jacobs[20], Inga Prokopenko[6,21], Nicole Soranzo[16,22], Toshiko Tanaka[23], Nicholas J Timpson[24], Peter Almgren[25], Amanda Bennett[26], Richard N Bergman[27], Sheila A Bingham[28,29], Lori L Bonnycastle[30], Morris Brown[31], Noël P Burtt[9], Peter Chines[30], Lachlan Coin[32], Francis S Collins[30], John M Connell[33], Cyrus Cooper[34], George Davey Smith[24], Elaine M Dennison[34], Parimal Deodhar[30], Paul Elliott[32], Michael R Erdos[30], Karol Estrada[20], David M Evans[24], Lauren Gianniny[9], Christian Gieger[7], Christopher J Gillson[4,5], Candace Guiducci[9], Rachel Hackett[9], David Hadley[13], Alistair S Hall[35], Aki S Havulinna[36], Johannes Hebebrand[37], Albert Hofman[38], Bo Isomaa[39], Kevin B Jacobs[40], Toby Johnson[41–43], Pekka Jousilahti[36], Zorica Jovanovic[5,44], Kay-Tee Khaw[45], Peter Kraft[46], Mikko Kuokkanen[9,47], Johanna Kuusisto[48], Jaana Laitinen[49], Edward G Lakatta[50], Jian'an Luan[4,5], Robert N Luben[45], Massimo Mangino[51], Wendy L McArdle[52], Thomas Meitinger[53,54], Antonella Mulas[17], Patricia B Munroe[55], Narisu Narisu[30], Andrew R Ness[56], Kate Northstone[52], Stephen O'Rahilly[5,44], Carolin Purmann[5,44], Matthew G Rees[30], Martin Ridderstråle[57], Susan M Ring[52], Fernando Rivadeneira[20,38], Aimo Ruokonen[58], Manjinder S Sandhu[4,45], Jouko Saramies[59], Laura J Scott[1], Angelo Scuteri[60], Kaisa Silander[47], Matthew A Sims[4,5], Kijoung Song[12], Jonathan Stephens[61], Suzanne Stevens[51], Heather M Stringham[1], Y C Loraine Tung[5,44], Timo T Valle[62], Cornelia M Van Duijn[38], Karani S Vimaleswaran[4,5], Peter Vollenweider[63], Gerard Waeber[63], Chris Wallace[55], Richard M Watanabe[64], Dawn M Waterworth[12], Nicholas Watkins[61], The Wellcome Trust Case Control Consortium[76], Jacqueline C M Witteman[38], Eleftheria Zeggini[6], Guangju Zhai[22], M Carola Zillikens[20], David Altshuler[9,10], Mark J Caulfield[55], Stephen J Chanock[8], I Sadaf Farooqi[5,44], Luigi Ferrucci[23], Jack M Guralnik[65], Andrew T Hattersley[66], Frank B Hu[14,15], Marjo-Riitta Jarvelin[32], Markku Laakso[48], Vincent Mooser[12], Ken K Ong[4,5], Willem H Ouwehand[16,61], Veikko Salomaa[36], Nilesh J Samani[51], Timothy D Spector[22], Tiinamaija Tuomi[67,68], Jaakko Tuomilehto[62], Manuela Uda[17], André G Uitterlinden[20,38], Nicholas J Wareham[4,5], Panagiotis Deloukas[16], Timothy M Frayling[19], Leif C Groop[25,69], Richard B Hayes[8], David J Hunter[9,14,15,46], Karen L Mohlke[70], Leena Peltonen[9,16,71], David Schlessinger[72], David P Strachan[13], H-Erich Wichmann[7,73], Mark I McCarthy[6,21,74,78,79], Michael Boehnke[1,78,79], Inês Barroso[16,78,79], Gonçalo R Abecasis[18,78,79] & Joel N Hirschhorn[3,11,75,78,79] for the GIANT Consortium[80]

Reflection on the study design

	Study 1 (EPIC-Norfolk subcohort) n=2269		Study 2 (EPIC-Norfolk obese set) n=1009		Study 3 (1958 British birth cohort) n=1375		Study 4 (CoLaus) n=5367		Study 5 (GEMS study) n=1665	
	β coeff (SE)	p value	β coeff (SE)	p value	β coeff (SE)	p value	β coeff (SE)	p value	β coeff (SE)	p value
rs4420638	0·24 (0·04)	$1·9\times10^{-9}$	0·14 (0·06)	0·02	0·25 (0·04)	$2·8\times10^{-9}$	0·05 (0·01)	$6·2\times10^{-12}$	0·04 (0·01)	$5·6\times10^{-3}$
rs599839	−0·15 (0·04)	$5·8\times10^{-5}$	−0·23 (0·06)	$7·6\times10^{-5}$	−0·14 (0·04)	$4·3\times10^{-4}$	−0·04 (0·01)	$1·6\times10^{-7}$	−0·06 (0·01)	$2·0\times10^{-5}$
rs4970834	−0·13 (0·04)	$1·1\times10^{-3}$	−0·18 (0·06)	$5·5\times10^{-3}$	−0·11 (0·04)	0·01	−0·04 (0·01)	$1·9\times10^{-6}$	−0·04 (0·01)	$2·8\times10^{-3}$
rs562338	−0·17 (0·04)	$6·0\times10^{-6}$	−0·11 (0·06)	0·07	−0·18 (0·05)	$1·1\times10^{-4}$	−0·03 (0·01)	$2·7\times10^{-6}$	−0·02 (0·01)	0·18
rs7575840	0·15 (0·03)	$6·3\times10^{-6}$	0·15 (0·05)	$2·4\times10^{-3}$	0·04 (0·04)	0·26	0·03 (0·01)	$1·9\times10^{-6}$	0·02 (0·01)	0·13
rs478442	−0·16 (0·04)	$2·1\times10^{-5}$	−0·07 (0·06)	0·25	−0·16 (0·04)	$3·6\times10^{-4}$	−0·03 (0·01)	$2·7\times10^{-5}$	−0·02 (0·01)	0·06
rs4591370	−0·17 (0·04)	$7·7\times10^{-6}$	−0·06 (0·06)	0·28	−0·16 (0·04)	$4·2\times10^{-4}$	−0·03 (0·01)	$3·2\times10^{-5}$	−0·02 (0·01)	0·06
rs4560142	−0·16 (0·04)	$1·6\times10^{-5}$	−0·06 (0·06)	0·27	−0·16 (0·04)	$4·2\times10^{-4}$	−0·03 (0·01)	$3·5\times10^{-5}$	−0·03 (0·01)	0·05
rs576203	−0·16 (0·04)	$1·2\times10^{-5}$	−0·07 (0·06)	0·25	−0·16 (0·04)	$3·5\times10^{-4}$	−0·03 (0·01)	$3·5\times10^{-5}$	−0·02 (0·01)	0·06
rs506585	−0·16 (0·04)	$1·7\times10^{-5}$	−0·06 (0·06)	0·31	−0·16 (0·04)	$3·5\times10^{-4}$	−0·03 (0·01)	$4·2\times10^{-5}$	−0·03 (0·01)	0·05
rs488507	−0·14 (0·04)	$1·3\times10^{-4}$	−0·07 (0·06)	0·25	−0·16 (0·04)	$3·3\times10^{-4}$	−0·03 (0·01)	$3·4\times10^{-5}$	−0·02 (0·01)	0·07
rs538928	−0·16 (0·04)	$5·0\times10^{-5}$	−0·01 (0·06)	0·92	−0·16 (0·04)	$3·5\times10^{-4}$	−0·03 (0·01)	$3·6\times10^{-5}$	−0·02 (0·01)	0·05
rs10402271	0·04 (0·03)	0·17	0·11 (0·05)	0·02	0·12 (0·04)	$7·5\times10^{-4}$	0·02 (0·01)	$5·2\times10^{-4}$	0·04 (0·01)	$8·3\times10^{-4}$
rs693	−0·12 (0·03)	$1·3\times10^{-4}$	−0·07 (0·05)	0·15	−0·06 (0·03)	0·06	−0·03 (0·01)	$1·0\times10^{-5}$	−0·02 (0·01)	0·16

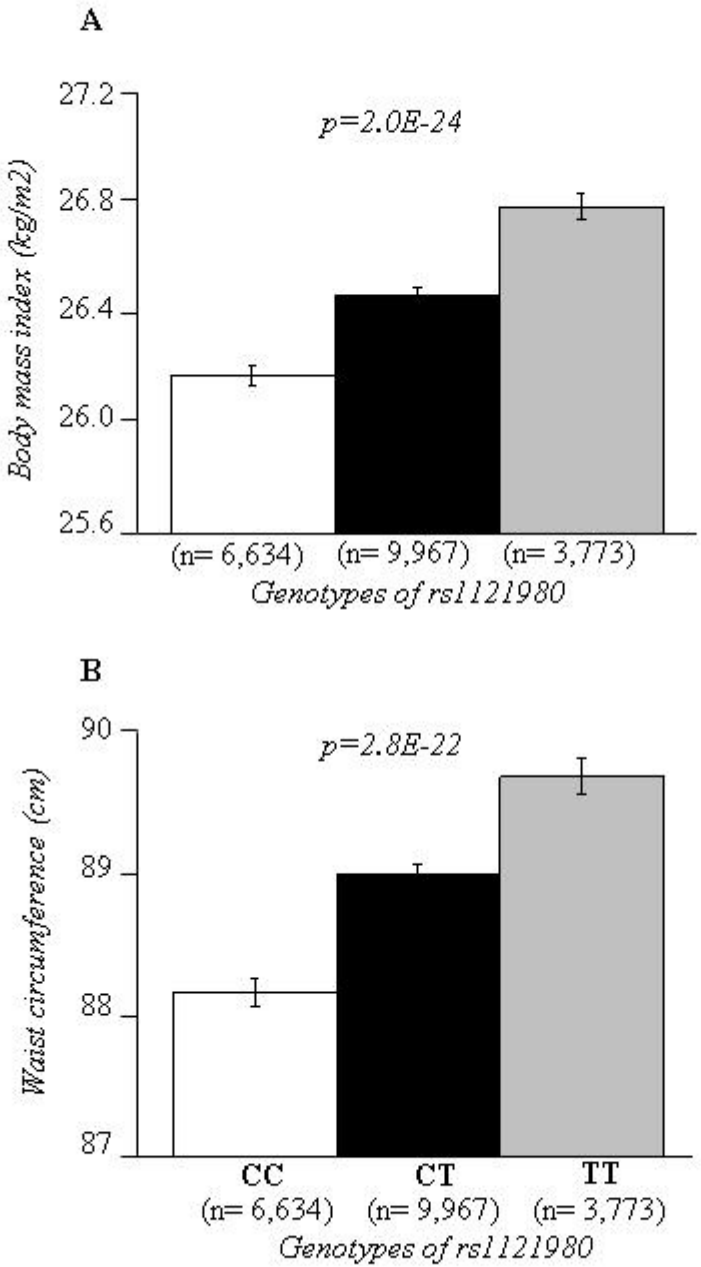
Webtable 3: Associations between Affymetrix SNPs with a combined p value of $<1·0\times10^{-7}$ and circulating concentrations of LDL cholesterol in independent study populations

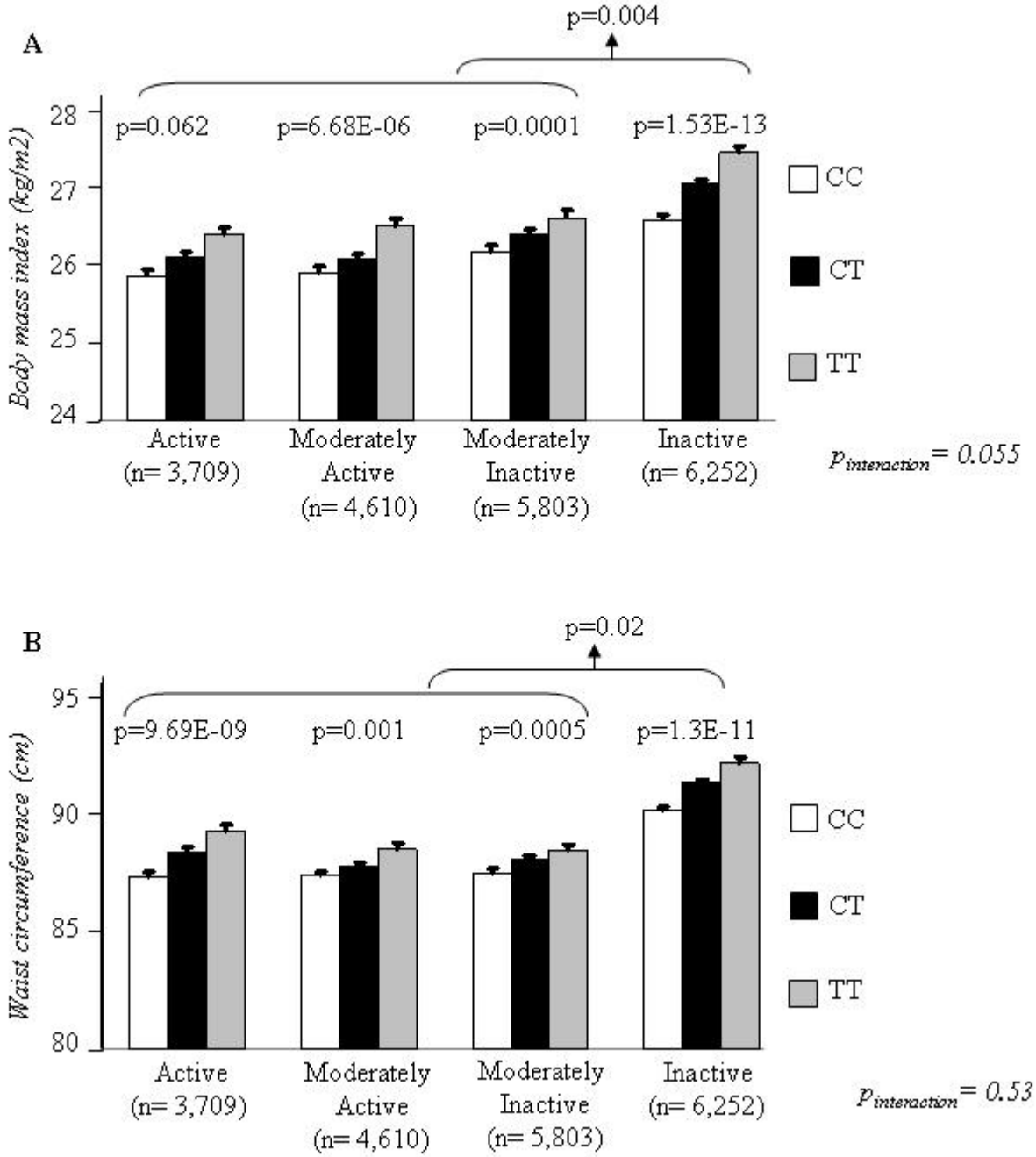
Current practice

- Linux clusters are now ready for comprehensive analyses and greatly facilitated by Linux/awk script which is light. awk proves very useful and can be transformed to Perl. In fact, any statistical package which processes data elements would be less efficient. An example is the transformation of long, wide, transposed format noted earlier. They call C/C++ programs such as IMPUTE/SNPTEST.
- We use Stata package to automate SNPTEST, and in some instances involved C/C++ code.
- SAS is still useful for data preparation, and in a sense less professional than DBMS such as Oracle but enjoys a large user community and has facility for data analysis.
- SAS 9.2 PROTO procedure allows for C/C++ to be called.

FTO/physical activity--BMI/WC association

- *FTO* variant, rs1121980, was genotyped in 20,374 participants (39-79 years) from the EPIC-Norfolk Study. Physical activity (PA) was assessed by a validated self-reported questionnaire. The interaction between rs1121980 and PA on BMI and waist circumference (WC) was examined by including the interaction term in mixed effect models.
- Our results show that PA attenuates the effect of *FTO* rs1121980 genotype on BMI and WC.


A
27.2
26.8
26.4
26.0
25.6
p=2.0E-24
Body mass index (kg/m2)
(n= 6,634)
(n= 9,967)
(n= 3,773)
Genotypes of rs1121980
B
90
89
88
87
p=2.8E-22
Waist circumference (cm)
CC
CT
TT
(n= 6,634)
(n= 9,967)
(n= 3,773)
Genotypes of rs1121980


A
p=0.004
p=0.062
p=6.68E-06
p=0.0001
p=1.53E-13
Body mass index (kg/m2)
28
27
26
25
24
CC
CT
TT
Active (n= 3,709)
Moderately Active (n= 4,610)
Moderately Inactive (n= 5,803)
Inactive (n= 6,252)
$p_{interaction}$= 0.055
B
p=0.02
p=9.69E-09
p=0.001
p=0.0005
p=1.3E-11
Waist circumference (cm)
95
90
85
80
CC
CT
TT
Active (n= 3,709)
Moderately Active (n= 4,610)
Moderately Inactive (n= 5,803)
Inactive (n= 6,252)
$p_{interaction}$= 0.53

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- Zhao JH. *J Stat Soft* 2007;23(8):1-18
- Zhao JH et al. *CCIS* 2007;2:781-90

II Association analysis

Topics

- Elements of association analysis
- Analytic tools
- R packages
- Examples
- Appendix

Elements of association analysis

- Quality control: call rates, Hardy-Weinberg equilibrium and minor allele frequencies and others such as clustering of genotypes, relatedness and population stratification.
- Test of associations
  - often through linear regression for continuous trait, and through logistic regression for binary, the proportion of variance explained for LR is measured through R^2 while the score statistic under additive model is equivalent to the Armitage trend test.
  - Genotype imputation: mostly often through HapMap CEU sample, involving ~2.5 million SNPs
  - Graphical presentation
- Interpretation, replication
- Report of findings

GRAMMAR

- It refers to genome-wide rapid association using mixed model and regression, and implemented in R/GenABEL. The method first obtains residuals adjusted for family effects and subsequently analyzes the association between these residuals and genetic polymorphisms using least-squares methods. It can also involves selected polymorphism to be followed up with the full measured genotype analysis (Aulchenko et al. Genetics 2007; 177:577-85).
- Initial model: $y_i = \mu + \Sigma_j \beta_j X_{ij} + G_i + e_i$
- We have the residuals $\hat{e}_i = y_i - (\hat{\mu} + \Sigma_j \hat{\beta}_j X_{ij} + \hat{G}_i) \equiv y_i *$
- Linear regression: $\hat{e}_i = \varphi + \gamma_i g_i + \varepsilon_i$
- Measured genotype model: $y_i = \mu + \gamma_i g_i + \Sigma_j \beta_j X_{ij} + G_i + e_i$
- The method adjusts for familial relationship, computationally fast, and ready to incorporate methods developed for “unrelated“ individuals in the second stage.

Graphics and association plots

- Plot of summary statistics
- Pedigree-drawing
- LD plot
- Q-Q plot -- contrasting observed versus expected log-p values
- Manhattan plot -- distribution of genome-wide p values
- Regional association plot -- including recombination, contribution from imputed SNPs and top hits from consortium meta-analysis

Analytical tools

- There are several reviews on *Human Genomics,* and an active list is maintained at http://linkage.rockefeller.edu
- LINKAGE, GENEHUNTER, Merlin, PAP, SAGE, SOLAR
- ETDT, EHPLUS, FBAT, QTDT, UNPHASED, SAS/Genetics
- R (genetics, gap, haplo.stats, haplin, kinship)
- For GWAS
  - HaploView
  - PLINK, SNPGWA
  - IMPUTE, MACH, BinBam
  - EIGENSTRAT
  - METAL
  - SAS, Stata, R (snpMatrix, GenABEL, SNPassoc)

Connections with R

- Occasionally, these software will be cross-referenced.
- Analyses with specialized programs such as IMPUTE/SNPTEST and PLINK are illustrated in the useR!2008 tutorial.
- snpMatrix provide connect with PLINK file, e.g., narac <- read.plink("narac.bed","narac.bim","narac.fam")

Basic R packages

- genetics
- haplo.stats
- gap
- Rassoc, HardyWeinberg, kinship, multic, pedigree, identity

- See CRAN task view for Genetics (http://cran.r-project.org/web/views/Genetics.html), as with an earlier review on the motivation for analysis with R statistical and computational environment (Zhao & Tan. *Hum Genomics* 2006;2:258-65) It also refers to Rgenetics projects whose packages are now available from http://www.bioconductor.org.

Hardy-Weinberg equilibrium tests

Suppose g is a data frame containing genotype counts for a list of SNPs. We can obtain exact HWE p value as follows.

```
library(gap)
head(g)
  comhom  het rarehom
1  12879 6699     961
2  13463 6214     799

for(i in 1:2) print(snp.HWE(as.numeric(g[i,])))
[1] 0.01843766
[1] 0.01542034
```

Gene-counting method: ABO blood type

```
library(VGAM)
abodat <- data.frame(A = 186, B = 38, AB = 13, O = 284)
fit <- vglm(cbind(A, B, AB, O) ~ 1, ABO, abodat)
fit
coef(fit)

Coefficients:
(Intercept):1 (Intercept):2
   -1.303414     -2.941384

Degrees of Freedom: 2 Total; 0 Residual
Residual Deviance: 0.3917573
Log-likelihood: -8.372631
> Coef(fit)
       pA         pB
0.21359094 0.05014533
```

Transmission/disequilibrium test (TDT)

```
library(gap)
x <- matrix(c(0,0, 0, 2, 0,0, 0, 0, 0, 0, 0, 0,
              0,0, 1, 3, 0,0, 0, 2, 3, 0, 0, 0,
              2,3,26,35, 7,0, 2,10,11, 3, 4, 1,
              2,3,22,26, 6,2, 4, 4,10, 2, 2, 0,
              0,1, 7,10, 2,0, 0, 2, 2, 1, 1, 0,
              0,0, 1, 4, 0,1, 0, 1, 0, 0, 0, 0,
              0,2, 5, 4, 1,1, 0, 0, 0, 2, 0, 0,
              0,0, 2, 6, 1,0, 2, 0, 2, 0, 0, 0,
              0,3, 6,19, 6,0, 0, 2, 5, 3, 0, 0,
              0,0, 3, 1, 1,0, 0, 0, 1, 0, 0, 0,
              0,0, 0, 2, 0,0, 0, 0, 0, 0, 0, 0,
              0,0, 1, 0, 0,0, 0, 0, 0, 0, 0, 0),nrow=12)
xx <- mtdt2(x,refcat="12")
```

- We obtain results similar to ETDT (Sham PC, Curtis D (1995) An extended transmission/disequilibrium test (TDT) for multi-allelic marker loci. Ann. Hum. Genet. 59:323-336).

Haplotype analysis

```
library(haplo.stats)
mc4r.map <- read.table("mc4r.map",as.is=TRUE)
snps <- mc4r.map[,2]
M <- length(snps)
a1 <- sprintf("%s%s",snps,rep(".a1",M))
a2 <- sprintf("%s%s",snps,rep(".a2",M))
a1a2 <- c(a1,a2)
for(i in 1:M) {a1a2[2*i-1] <- a1[i];a1a2[2*i] <- a2[i]}
mc4r <-
    read.table("mc4r.ped",col.names=c(paste("v",1:6,sep=""),a1a2))

pheno <- read.csv("mc4r.csv",sep="\t",skip=11)
cohort <- subset(pheno,cohort==1)
attach(cohort)
mc4r.12 <-
    haplo.score(bmi,mc4r[id,7:30],x.adj=sex+age,locus.label=snps[1:12
    ])
```

Generalized linear models

```
mc4r.geno <-
    setupGeno(mc4r[id,7:dim(mc4r)[2]],locus.label=snps)
attr(mc4r.geno,"unique.alleles")[1:12]
mc4r.12 <-
    haplo.score(bmi,mc4r.geno[,1:24],x.adj=sex+age,locus.label
    =snps[1:12])


mc4r.data <- data.frame(geno=mc4r.geno,cohort)
mc4r.gauss <- haplo.glm(bmi ~ sex + age + geno, family =
    gaussian, na.action="na.geno.keep",
    allele.lev=attributes(geno)$unique.alleles,
    data=mc4r.data, locus.label=snps,
    control = haplo.glm.control(haplo.freq.min=0.02))
mc4r.gauss
detach(cohort)
```

Gene-gene, gene-environment interaction

- haplo.glm is considerably slower but it is among the few facilities for GEI analysis
- A recent analysis of *SNCA-LRRK2* interaction with Parkinson's disease

```
snca_assign <- read.dta("snca_post.dta")
snca_lrrk2_int1 <-
    haplo.glm(formula=y~ssex+snca_assign$hap12*lrrk
    2,family="binomial",data=clean,locus.label=s2)
snca_lrrk2_int1
```

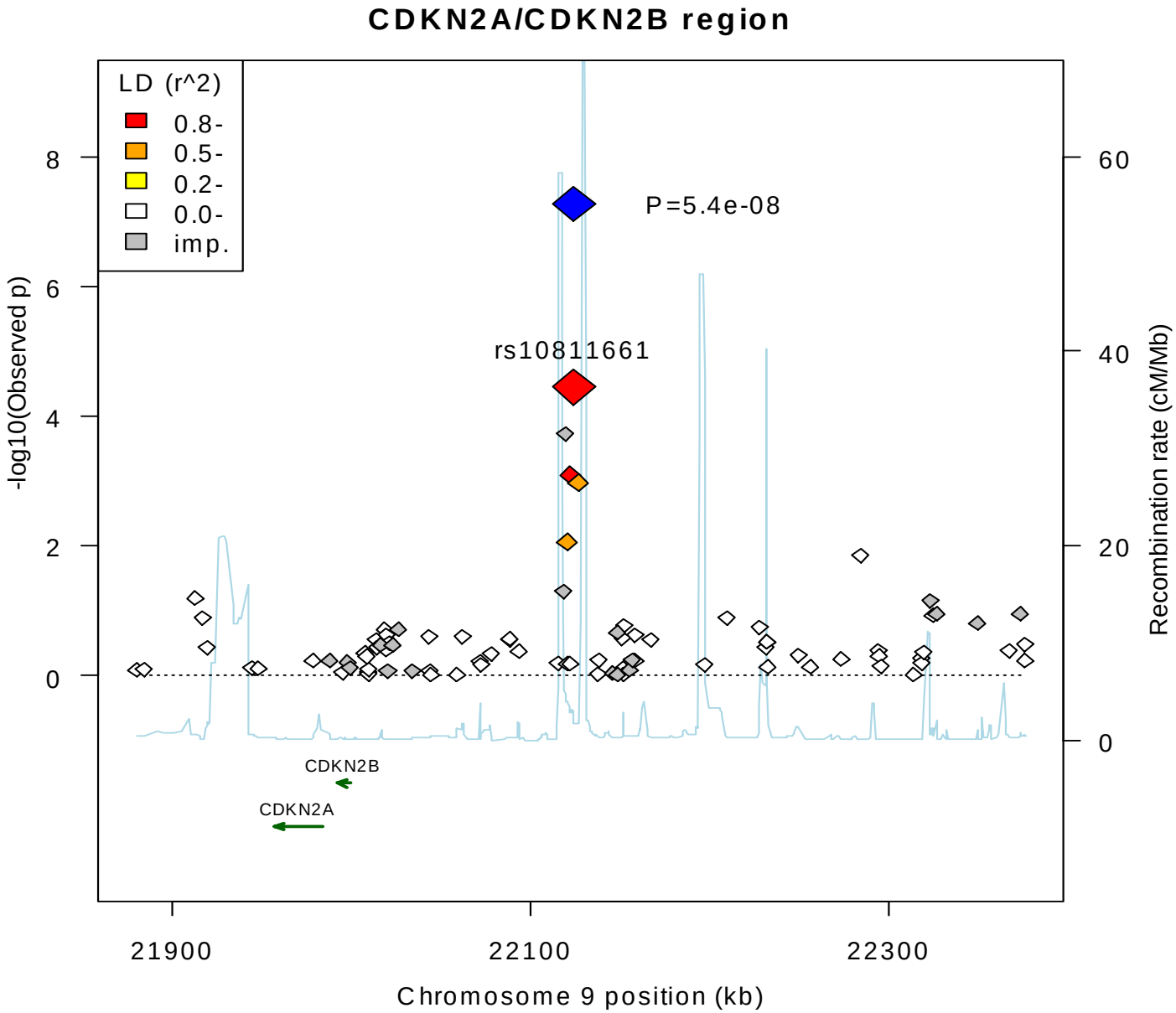
- Object snca_assign contains effective haplotype assignment based on *SNCA* and used as covariate for *SNCA-LRRK2* interaction analysis. We can also use *haplo.interaction* from SNPassoc.

Adjustment for multiple testing

```
Bonferroni.sig(wga, model="log-add",alpha=0.05)
library(qvalue)
q <- qvalue(p)
plot(q)
library(multtest)
adj <-
   c("Bonferroni","Holm","Hochberg","SidakSS","SidakSD",
   "BH","BY")
mt <- mt.rawp2adjp(p,adj)
mt.reject(cbind(mt$rawp,mt$adjp),seq(0,0.1,0.001))$r
```

Manhattan and regional association plots

```
library(gap)
# for the Framingham data analysis
png("figures.pdf")
par(mfrow=c(2,1),mai=c(1,1,0.2,0.8),ps=7)
qqunif(test$np,bg="blue",bty="n",xlim=c(0,6),cex=0.02)
par(las=2)
mhtplot(test,usepos=TRUE,pch=21,colors=rep(c("blue","green"),
    11),cutoffs=c(4,5,6),cex=0.02)
dev.off()
# DGI example for asplot
asplot("rs10811661", "CDKN2A/CDKN2B region", "9",
    CDKNlocus, CDKNmap, CDKNgenes, 5.4e-8, c(3,6))
```



Association plot

R packages for GWAS

- SNPassoc
- GenABEL
- P2BAT
- snpMatrix

- While the first three are available from CRAN, snpMatrix is available from BioConductor.
- Other packages include, multtest, meta, rmeta, CAMAN, qvalue, ROCR.

Setup for GWAS

- CRAN
  http://cran.r-project.org/web/packages/index.html
  > setRepositories()
  > install.packages(c(“SNPassoc”, “GenABEL”))
  > library(GenABEL)

- BioConductor
  > source("http://bioconductor.org/biocLite.R")
  > biocLite("snpMatrix")
  > library(snpMatrix)

Notes on S4 class

- We illustrate with two classes
  > library(snpMatrix)
  > showClass("snpMatrix")
  > library(GenABEL)
  > showClass("scan.gwaa")

- It is more informative with the following commands
  > class?snpMatrix
  > class?scan.gwaa

- Later we will omit the command prompt (>). We will also give examples of creating object with new() function.

Example – *MC4R* SNPs and BMI

- To make a smooth exposition we use our study of SNPs near *MC4R* and body mass index as reported by Loos et al. *Nat Genet* 2008;40:768-775.
- The *MC4R* gene is located on chromosome 18 and we will focus on SNPs rs17782313 and rs17700633 at positions 56002077 and 57000671 according to NCBI build 35, all genotypes being on forward strand.
- These were based on 3850 population-based individuals at stage 1 of the case-cohort study from which 3552 individuals remained after quality controls.
- We will run through SNPassoc, snpMatrix and GenABEL packages on data as contained in files mc4r.ped, mc4r.map and mc4r.csv

SNPassoc

```
library(SNPassoc)
map <- read.table("mc4r.map",sep="\t",as.is=TRUE)
info <- data.frame(snp=map[2],chr=map[2],pos=map[4])
ped <- read.table("mc4r.ped",sep="\t",as.is=TRUE)
names(ped) <- c(paste("v",1:6,sep=""),map[,2])
pheno <-
   read.csv("mc4r.csv",sep="\t",skip=11,header=TRUE,as.is=TR
   UE)
is.cohort <- pheno$cohort==1
cohort <- subset(pheno,is.cohort)
snp <- ped[,-c(1:6)][is.cohort,]
snps <- dim(snp)[2]
for(i in 1:snps)
{
   substr(snp[,i],2,2) <- "/"; empty <- (snp[,i]=="0/0");
   snp[empty,i] <- NA
}
```

Analysis

```
mc4r <- setupSNP(snp,1:snps,sep="/",sort=TRUE,info=info)
summary(mc4r)
plot(mc4r$rs17782313)
plot(mc4r$rs17700633,type=pie)
hwe <- tableHWE(mc4r)
mc4r.ld <- LD(mc4r)
summary(mc4r.ld)
mc4r.ld$"R^2"
attach(cohort)
association(bmi ~ sex+age+rs17782313,data=mc4r)
wga <- WGassociation(bmi ~ sex+age+1,model="log-
   add",data=mc4r)
png("mc4r.png")
qqpval(wga$"log-additive")
dev.off()
```

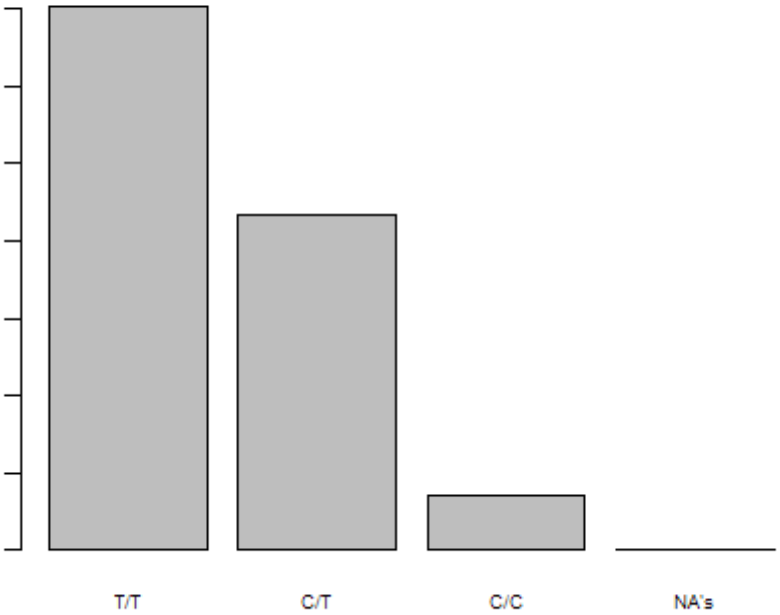
Summary statistics for two SNPs

mc4r$rs17782313

	frequency	percentage
C	1150	23.81
T	3680	76.19
NA's	4	NA

	frequency	percentage
T/T	1405	58.18
C/T	870	36.02
C/C	140	5.80
NA's	2	NA

HWE (pvalue): 0.736476

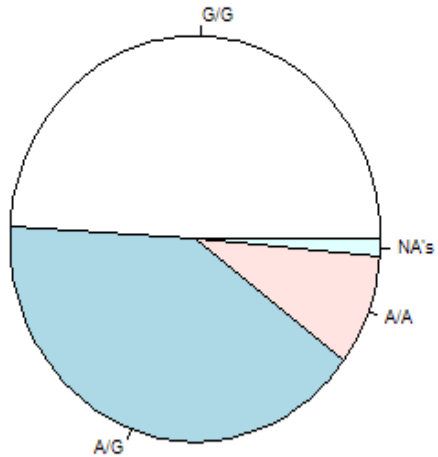


mc4r$rs17700633

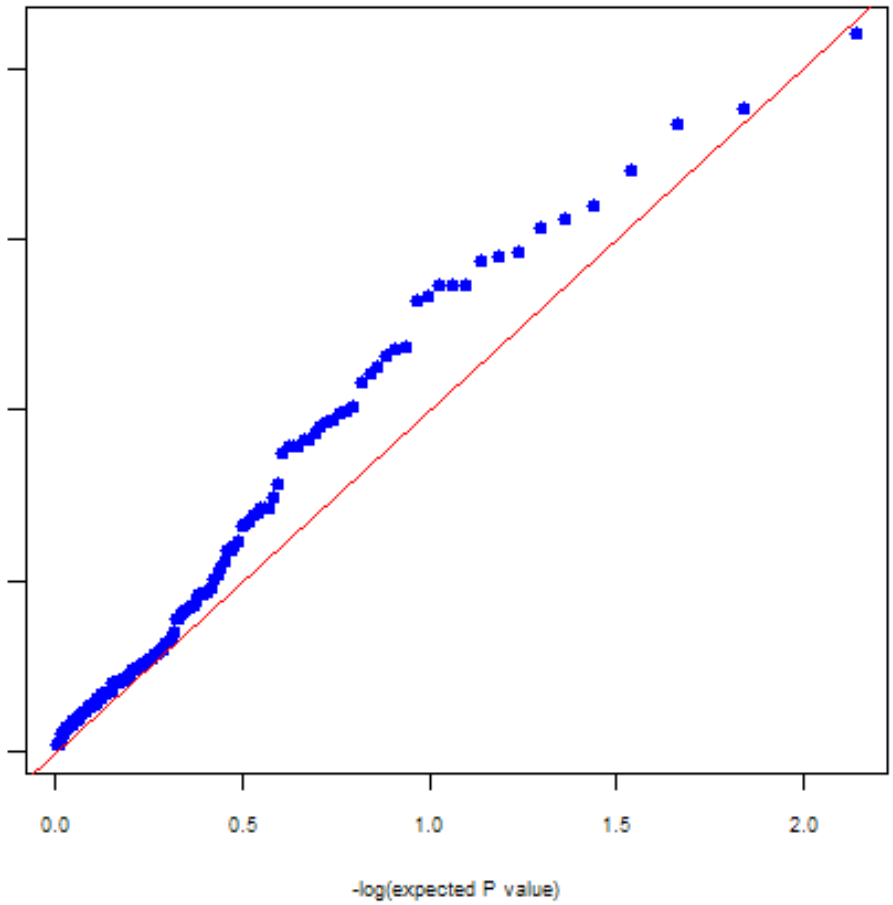
	frequency	percentage
A	1411	29.62
G	3353	70.38
NA's	70	NA

	frequency	percentage
G/G	1183	49.66
A/G	987	41.44
A/A	212	8.90
NA's	35	NA

HWE (pvalue): 0.768142



Q-Q plot



Comments

- SNPassoc is essentially designed for dealing with unrelated individuals but with considerable enhancements from genetics and haplo.stats.
- It implements permutation tests for binary traits through scanWGassociation(,nperm=) and permTest()
- It is possible to conduct gene-gene interaction:

```
mc4r.ip <-
   interactionPval(bmi~sex+age,data=mc4r,model="lo
   g-add")
plot(mc4r.ip)
```

- We got a very good feel of the kind of analysis it may involve and this is a very simple example.

snpMatrix

```
library(snpMatrix)
mc4r <- read.snps.pedfile("mc4r.ped")
summary(mc4r)
mc4rsnps <- row.names(mc4r$snp.support)
head(mc4r$snp.support)
head(mc4r$subject.support)
# quality controls
mc4r.qc <- summary(mc4r$snp.data)
head(mc4r.qc)
mc4r.ld <- ld.snp(mc4r$snp.data)
plot.snp.dprime(mc4r.ld,"mc4r.eps",scheme="rsq")
# ps2pdf mc4r.eps
# xpdf mc4r.pdf
# LD(rs17782313, rs17700633)
mc4r$snp.support[c(1,12),]
pair.result.ld.snp(mc4r$snp.data,1,12)
```

PCA and identity-by-state analysis

```
# PCA
mc4r.xxt <- xxt(mc4r$snp.data,correct.for.missing=TRUE)
mc4r.pc <- eigen(mc4r.xxt, symmetric=TRUE)
loadings <- snp.cor(mc4r$snp.data, mc4r.pc$vectors[,1:10])
# identity-by-state analysis
mc4r.ibs <- ibs.stats(mc4r$snp.data)
mc4r.count <- ibsCount(mc4r$snp.data)
mc4r.dist <- ibsDist(mc4r.count)
mc4r.clust <- hclust(mc4r.dist)
plot(mc4r.clust)
```

Note this is based on XX^T, in an order of N^2, where X and N are the genotype data matrix and number of individuals (see vignette for details).

Phenotype data and case-control analysis

```
# Phenotype data
pheno <- read.csv("mc4r.csv",skip=11,sep="\t")
pheno$cc<-ifelse(pheno$bmi>=30,1,0)
attach(pheno)

# Case-control analysis of all individuals
cc.test <- single.snp.tests(cc,snp.data=mc4r$snp.data)
summary(cc.test)
class(cc.test)
showClass("snp.tests.single")
chi.squared(cc.test,1) #qq.chisq(cc.test@chisq[,1])
```

Meta-analysis

```
# a meta-analysis
cc.test <-
   single.snp.tests(cc,snp.data=mc4r$snp.data,score=TRU
   E)
cc.test2 <- pool(cc.test,cc.test)
summary(cc.test2)
cc.test.sign <- effect.sign(cc.test)
table(cc.test.sign)
cc.test.sign[1:12]
cc.test.switch <- switch.alleles(cc.test,c(1,12))
effect.sign(cc.test.switch)[1:12]
```

OLS estimation and retrospective analysis

```
# ordinary least squares estimates
reg.rhs <-
    snp.rhs.tests(bmi~sex+age,family="gaussian",subset=(cohort==1),s
    np.data=mc4r$snp.data)
class(reg.rhs)
showClass("snp.tests.glm")
reg.rhs@df
qq.chisq(reg.rhs@chisq)
print(reg.rhs)

# retrospective models
reg.lhs <-
    snp.lhs.tests(mc4r$snp.data,~bmi,~sex+age,subset=(cohort==1))
class(reg.lhs)
showClass("snp.tests.glm")
reg.rhs@df
qq.chisq(reg.lhs@chisq,df=2)
```

Genotype imputation

- It is customarily to impute genotypes in a large study based on a small sample of fully-genotyped individuals, e.g., hapmap, so as to conduct association tests for large number of SNPs.
- It is also useful for meta-analysis of SNPs from different platforms such as Affymetrix 500K and Illumina 550K.
- As it is snpMatrix implements genotype imputation between sets of markers based on same individuals; more generally this involves genotypes from HapMap.

Hapmap and imputation

```
# ideally we would use 60/90 founders and a combination of hapmap
    CEU and our study sample
url.p1 <- "ftp://ftp.hapmap.org/hapmap/genotypes"
url.p2 <- "/latest_ncbi_build35/fwd_strand/non-redundant/"
url.p3 <- "genotypes_chr18_CEU_r21a_nr_fwd.txt.gz"
hapmap <- paste(url.p1,url.p2,url.p3,sep="")
chr18 <- read.HapMap.data(hapmap)
chr18snps <- row.names(chr18$snp.support)
summary(chr18)
sel <- chr18snps%in%mc4rsnps
impute.from <- chr18$snp.data[,!sel]
impute.to <- chr18$snp.data[,sel]
pos.from <- chr18$snp.support$Position[!sel]
pos.to <- chr18$snp.support$Position[sel]
mc4r.imp <- snp.imputation(impute.from, impute.to, pos.from, pos.to)
summary(mc4r.imp)
plot(mc4r.imp)
```

QC for chromosome X

```
# we omit the X.ped data/map here owing to their size
X <- read.snps.pedfile("X.ped",X=TRUE)
X.qc <- summary(X$snp.data)
X.col <- col.summary(X$snp.data)
SNPs <- subset(X.col,
    Call.rate>=0.90&MAF>=0.01&z.HWE>=1e-6)
write.csv(row.names(SNPs), "X.snps", quote=FALSE,
    row.names=FALSE)
library(foreign)
write.dta(X.col,"Xqc.dta")
```

By default, X.map is called which contains lines as follows

```
X     SNP_A-1787762  0     148021903
X     SNP_A-1788139  0     135986846
X     SNP_A-1789223  0     5694766
...
```

snp.imputation

- It is notable with the definition of snp.imputation that given two set of SNPs typed in the same subjects, this function calculates regression equations which can be used to impute one set from the other in a subsequent sample.
- We customarily use external data (e.g., available from HapMap, 1000 genomes or elsewhere) and our sample jointly, treating non-typed SNPs as missing.
- CRAN packages such as mice should facilitate this on the phenotype side.

Comments

- snpMatrix has explicit treatment of chromosome X. It also provides some facilities for dealing with family data. The retrospective method would be more appropriate with data involving the kind of sample selection here. Please check for snpMatrix vignette for use of hexbin package.
- It is possible to take advantage of the S4 class facility as implemented in the package when coded genotypes are available from or to other sources, e.g.,

```
m1 <- new('snp.matrix',dm1)
m2 <- new('snp.matrix',dm2)
m <- snp.rbind(m1,m2)
write.snp.matrix(m,"m.dat")
```

GenABEL: Flowchart (Q Zhang from WUSTL)

gwaa.data-class

phdata:
phenotypic data
(data frame)

gtdata:
genotypic data
(snp.data-class)

snp.data()

nbytes: number of bytes used to store data on a SNP
nids: number of people
male: male code
idnames: ID names
nsnps: number of SNPs
nsnpnames: list of SNP names
chromosome: list chromosomes corresponding to SNPs
coding: list of nucleotide coding for SNP names
strand: strands of the SNPs
map: list SNPs' positions
gtps: genotypes (snp.mx-class)

2-bit storage
0 00
1 01
2 10
3 11
Save 75%

load.gwaa.data(phenofile = "pheno.dat", genofile = "geno.raw")

```
id sex age bt1 qt qt1
"289982" 0 30.33 NA NA 3.93
"325286" 0 36.514 1 0.49 3.61
"357273" 1 37.811 0 1.65 5.30
"872422" 1 20.393 0 1.95 4.07
"1005389" 1 28.21 1 0.35 3.90
```

```
289982 325286 357273 872422 1005389
SNP-1886933 SNP-2264565 SNP-2305014
1 1 1
825852 2137143 2585920
3 3 3 3 2
3 2 3 3 3
2 2 1 1 1
```

- convert.snp.text() from text file (GenABEL default format)
- convert.snp.ped() from Linkage, Merlin, Mach, and similar files
- convert.snp.mach() from Mach format
- convert.snp.tped() from PLINK TPED format
- convert.snp.illumina() from Illumina/Affymetrix-like format

Data manipulation

- snp.subset: subset data by snp names or by QC criteria
- add.phdata: merge extra phenotypic data to the gwaa.data-class.
- ztransform: standard normalization of phenotypes
- rntransform: rank-normalization of phenotypes
- npsubtreated: non-parametric adjustment of phenotypes for medicated subjects

QC and summary statistics

- summary.snp.data: summary of snp data (Number of observed genotypes, call rate, allelic frequency, genotypic distribution, P-value of HWE test
- check.trait: summary of phenotypic data and outlier check based on a specified p/FDR cut-off
- check.marker: SNP selection based on call rate, allele frequency and deviation from HWE
- HWE.show: showing HWE tables, Chi2 and exact HWE P-values
- perid.summary: call rate and heterozygosity per person
- ibs: matrix of average IBS for a group of people & a given set of SNPs
- hom: average homozygosity (inbreeding) for a set of people, across multiple markers

SNP association scans

- scan.glm performs snp association test, e.g., scan.glm((“y~x1+x2+...+CRSNP", family = gaussian(), data, snpsubset, idsubset).
- scan.glm.2D:2-snp interaction scan.
- ccfast: case-control association analysis by computing chi-square test from 2x2 (allelic) or 2x3 (genotypic) tables. emp.ccfast obtains Genome-wide significance (permutation) for ccfast scan.
- qtscore: association test (GLM) for a trait (quantitative or categorical) emp.qtscore() is genome-wide significance (permutation) for qscore() scan.
- mmscore: score test for association between a trait and genetic polymorphism, in samples of related individuals (needs stratification variable, scores are computed within strata and then added up).
- egscore: association test, adjusted for possible stratification by principal components of genomic kinship matrix (snp correlation matrix).

Haplotype association scans

- scan.haplo: haplotype association test using GLM in R library
- scan.haplo.2D: 2-haplotype interaction scan

Results as in scan.gwaa class

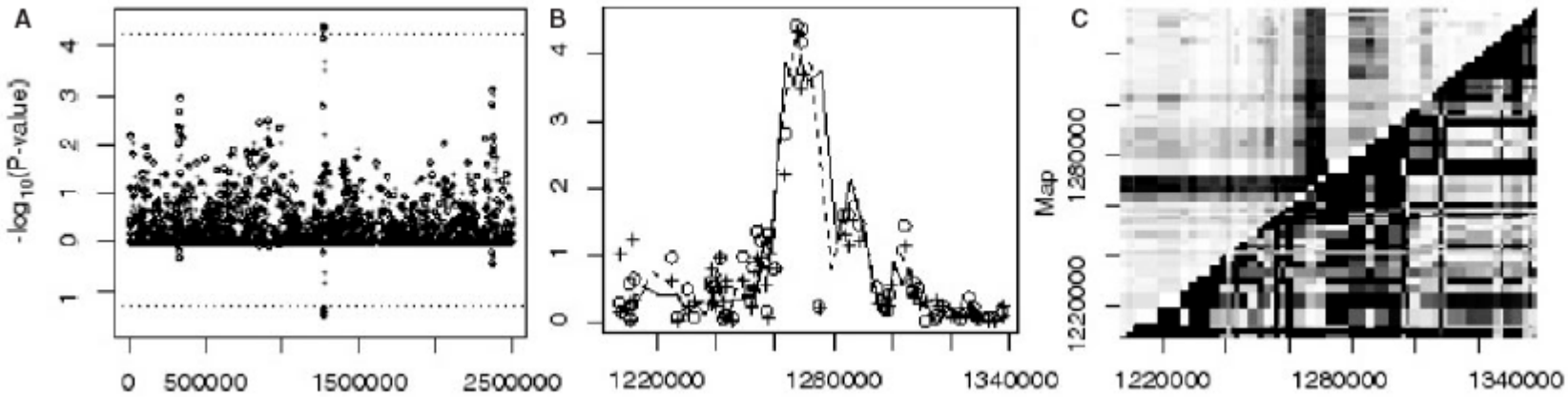
from scan.glm, scan.haplo, ccfast, qtscore, emp.ccfast,emp.qtscore

- Names: snpnames list of names of SNPs tested
- P1df: p-values of 1-d.f. (additive or allelic) test for association
- P2df: p-values of 2-d.f. (genotypic) test for association
- Pc1df: p-values from the 1-d.f. test for association between SNP and trait; the statistics is corrected for possible inflation
- effB: effect of the B allele in allelic test
- effAB: effect of the AB genotype in genotypic test
- effBB: effect of the BB genotype in genotypic test
- Map: list of map positions of the SNPs
- Chromosome: list of chromosomes the SNPs belong to
- Idnames: list of subjects used in analysis
- Lambda: inflation factor estimate, as computed using lower portion (say, 90%) of the distribution, and standard error of the estimate
- Formula: formula/function used to compute p-values
- Family: family of the link function / nature of the test

Table and graphics

- descriptives.marker(): table of marker info.
- descriptives.trait(): table of trait info.
- descriptives.scan(): table of scan results

- plot.scan.gwaa(): plot of scan results
- plot.check.marker(): plot of marker data (QC etc.)



ParallABEL

- An R Library for Generalized Parallelization of Genome-Wide Association Studies

- http://parallabel.r-forge.r-project.org/
- http://www.sci.psu.ac.th/units/genome/CGBR/ParallABEL/index.html
- Sangket et al. BMC Bioinformatics 2010; 11:217

Applied to *MC4R* data

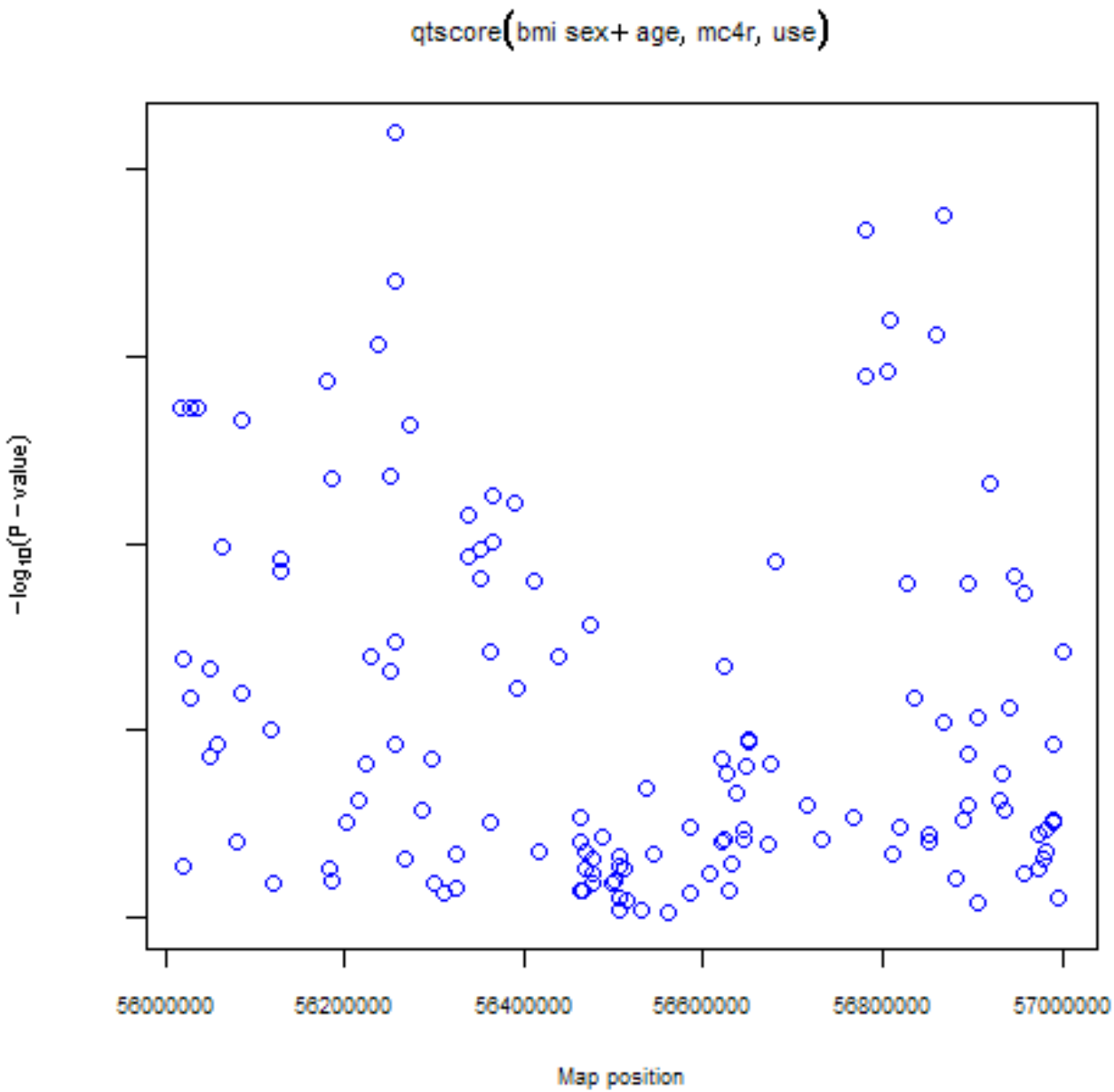
```
library(GenABEL)
convert.snp.ped("mc4r.ped","mc4r.map2","mc4r.out",strand="+
    ")
csv <- read.csv("mc4r.csv",skip=11,sep="\t",as.is=TRUE)
attach(csv)
csv2 <- data.frame(id,sex=2-sex,cohort,age,bmi,zbmi,rbmi)
write.table(csv2,"mc4r.csv2",sep=" ",row.names=FALSE)
mc4r <- load.gwaa.data(phe = "mc4r.csv2", gen = "mc4r.out",
    force = TRUE)
```

Note that the map2 file now has three columns: chromosome, SNP names and positions. It also explicitly allow for strand. The addition of phenotypic information is via the load.gwaa.data, which requires specification of id and sex (0=female, 1=male) in a strictly way.

Analysis

```
HWE.show(mc4r)
r2 <- r2fast(mc4r)
dp <- dprfast(mc4r)
rho <- rhofast(mc4r)
descriptives.trait(mc4r)
descriptives.marker(mc4r)
use <- csv$cohort==1
qt.bmi <- qtscore(bmi~sex+age,data=mc4r,idsubset=use)
plot(qt.bmi)
```

- However, as is shown here once the gwaa.data is defined a range of analyses can be rather straightforward.
- Again we only focus on the cohort sample (cohort==1).



Scatter plot of p values

GAW15 Expression quantitative trait

- There is substantial individual variation in expression level of genes, which is smaller in monozygotic twins than among individuals of other relationships, suggesting a genetic component (Morley M, Molony CM, Weber TM, Devlin JL, Ewens KG, Spielman RS, Cheung VG: Genetic analysis of genome-wide variation in human gene expression. *Nat* 2004, 430:743-747).
- Genetic Analysis Workshop 15 problem 1 provided
  - 14 three-generation families
  - 2554 expression quantitative traits
  - 2882 SNP genotypes
  - Chromosomal positions of these SNPs
- These information was contained in comma-delimited files each with appropriate header. This simple example serves to illustrate the basic analysis involved.

Getting data into R

- We first got data into R,

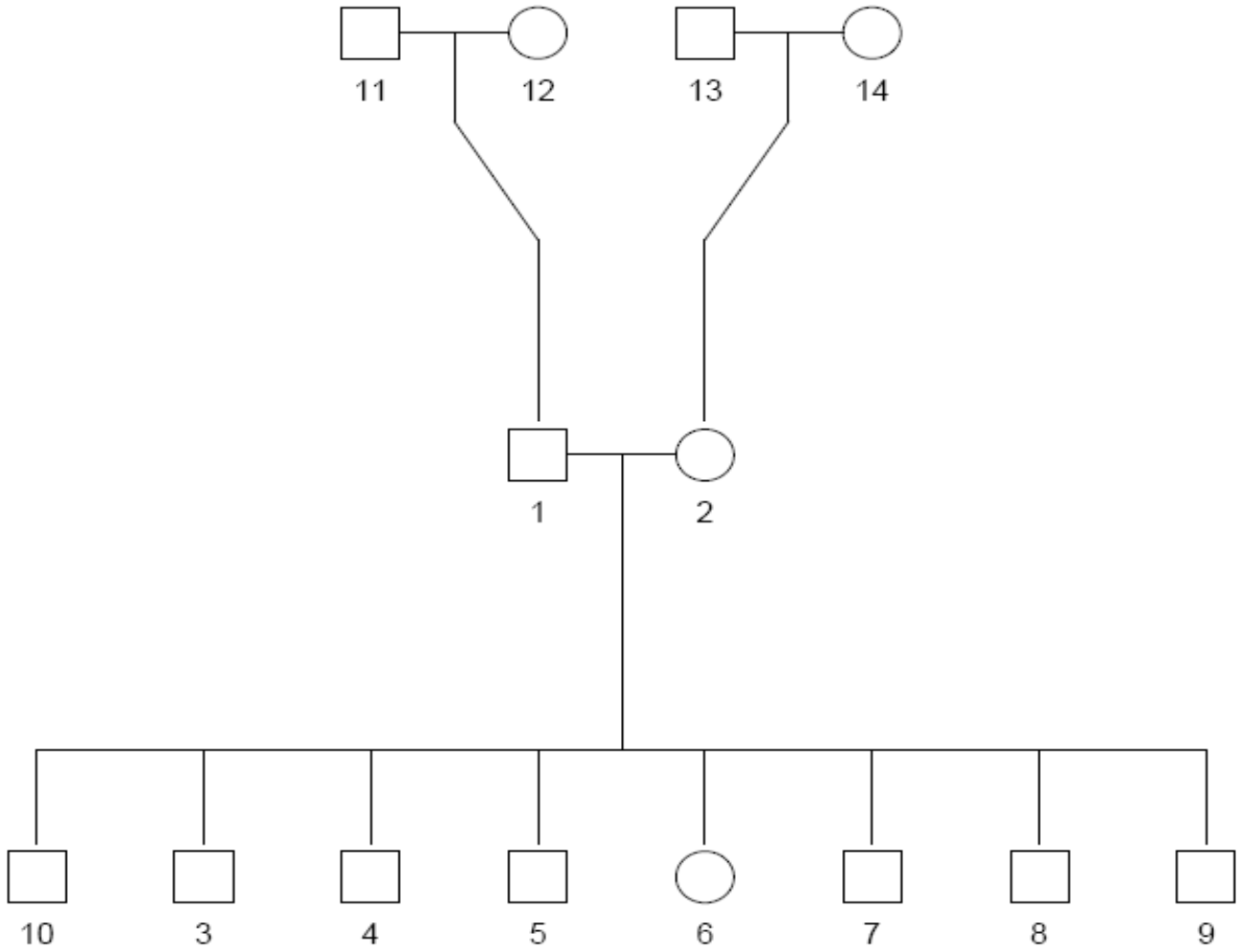
```
id <- read.table("LINKAGE.PED",header=T,as.is=T,sep=",")
phn <-
   read.table("LINKAGE.PHN",header=T,as.is=T,sep=",")
snp <-
   read.table("LINKAGE.SNP",header=T,as.is=T,sep=",",na.st
   ring="0/0")
map <-
   read.table("LINKAGE.MAP",header=T,as.is=T,sep=",")
pheno <- merge(id,phn,by=c("FAMID","ID"))
ped <- merge(pheno,snp,by=c("FAMID","ID"))
```

- Now the object ped has all the necessary information. We omit details of association testing but pedigree diagrams.

Pedigree diagrams

```
library(kinship)
pdf("pedfile.pdf"); attach(ped)
uid <- unique(ped$FAMID)
for (j in 1:length(uid))
{
  selected <- FAMID==uid[j]
  id <- ID[selected]
  dadid <- FA[selected]
  momid <- MO[selected]
  sex <- SEX[selected]
  par(xpd=TRUE)
  ped <- pedigree(id, dadid, momid, sex)
  plot(ped, id=paste("\n",id,sep=""))
  title(uid[j])
  k <- kinship(id,dadid,momid)
  print(k)
}
detach(ped); dev.off()
```

A typical pedigree diagram



GAW16 Framingham data

- Data management through SAS
- QC and basic association statistics via PLINK
- Estimation of inflation factor by snpMatrix
- Cross-check with GRAMMAR procedure from R/GenABEL

```
library(GenABEL)
# this is an example of Framingham data for GAW16
convert.snp.tped(tped = "chrall.tped", tfam = "pheno.tfam",
   out = "chrall.raw", strand = "+")
df <- load.gwaa.data(phe = "pheno.dat", gen = "chrall.raw",
   force = TRUE)
```

- Longitudinal data with SAS, Stata and Mplus. Rpackages include gee, nlme and packages which handle family data, e.g., kinship, GWAF, pedigreemm.
- Graphics via R/gap

References

- Elston RC. Introduction and overview. *Stat Meth Med Res* 9(6, special issue), 2000
- Balding DJ. *Nat Rev Genet* 7:781-791, 2006
- Elston RC, Anne Spence M. *Stat Med* 25:3049-3080, 2006
- McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JPA, Hirschhorn JN. Genome-wide association studies for complex traits: Consensus, uncertainty and challenges. *Nat Rev Genet 9:*356–369, 2008
- Zheng G, Marchini J, Geller NL. Introduction to the special issue: Genome-wide association studies. *Stat Sci* 24(4, special issue), 2009

Appendix- GWAS with SAS and Stata

- Procedures in SAS/BASE and other modules provide graphics, database support and internet connectivity.
- SAS/STAT provides standard procedures including linear and logistic regressions or generalized linear (nonlinear, mixed) model as well as covariance and linear structure model (CALIS), MULTTEST.
- SAS/Genetics includes procedures for summarizing marker data (ALLELE), inferring and tagging haplotypes (HAPLOTYPE and HTSNP), association testing in population-based (CASECONTROL) and family-based (FAMILY) samples.

The transposed data format

rs17782313	TT	CT	TT	TT	TT	TT	TT	CC
rs8097644	CC	CC	AC	CC	CC	CC	CC	CC
rs9947403	CC	TT	CC	CC	CC	CC	CC	TT
rs639407	AA	GG	AA	AA	AA	AA	AA	GG
rs11665563	CC	CT	CC	CC	CC	CC	CC	TT
rs11663816	TT	CT	TT	TT	TT	TT	TT	CC
rs619662	GG	AA	AG	GG	GG	GG	GG	AA
rs727406	GG	GG	GT	GG	0	0	GG	GG
rs8089366	GG	GT	GG	GG	GG	GG	GG	TT
rs11152217	GG	GT	GG	GG	GG	GG	GG	GG
rs9955666	GG	AG	GG	GG	GG	GG	GG	AA
rs17700633	GG	AG	GG	GG	GG	GG	AG	AA
rs9946888	TT	CT	CT	CT	TT	CT	CT	TT
rs9961245	CC	CT	CT	CT	CC	CT	CT	CC
rs17066774	GG	GG	GG	GG	GG	GG	GG	GG

Data preparation

```
data long (keep=&snpid id &vlist a1a2 add n);
      set data; fid=open("data");
      length id $11. add 3. a1a2 $3.; format add 1.;
      set map point=_n_;
      n=0;
      do col=2 to attrn(fid,"nvars");
          iid=col-1;
          set &trait (keep=&vlist) point=iid;
          if &inc=1 then do;
            id=varname(fid,col); a1a2=vvaluex(id);  add=.;
            if a1a2 ne "   " then do;
              a1=substr(a1a2,1,1); a2=substr(a1a2,3,1);
              add=(a1=b)+(a2=b);
              n+1;
            end; output;
          end;
      end; rc=close(fid);
run;
```

Analysis

```
ods select none;
proc allele data=long genocol;
      by rsn notsorted;
      var a1a2;
      ods output markersumm=ms allelefreq=out.af;
run;
proc reg data=long;
      by rsn notsorted;
      ods output parameterestimates=bmipm;
      model bmi = age add / b stb;
quit;
proc logistic data=long descending;
      by rsn notsorted;
      ods output parameterestimates=obpm CLOddsPL=obclpm;
      model obesity = age add / expb clodds=pl;
run;
```

Stata

- It is a general-purpose, modern and easy to use statistical analysis system (e.g., http://en.wikipedia.org/wiki/Stata).
- Functions for genetic data includes summary statistics, test of Hardy-Weinberg equilibrium, haplotype estimation, tagging and association analysis.
- It allows for C/C++ routines to be used for computer intensive tasks. My colleague has implemented SNPTEST-based GWA analysis to automate a variety of sample and analyses for imputed genotypes.
- There is also a good implementation for meta-analysis (metan, etc), as with a set of functions for instrumental variable regressions in our context.

Programs by David Clayton

- ginsheet- Read genotype data from text files.
- gloci - Make a list of loci.
- greshape - Reshape a file containing genotypes to a file of alleles.
- gtab - Tabulate allele frequencies within genotypes and generate indicators (performs Hardy-Weinberg Equilibrium testing).
- gtype - Create a single genotype variable from two allele variables.
- htype - Create a haplotype variable from allele variables.
- mltdt - Multiple locus TDT for haplotype tagging SNPs (htSNPs).
- origin - Analysis of parental origin effect in TDT trios.
- pseudocc - Create a pseudo-case-control study from case-parent trios.
- pscc - Experimental version of pseudocc in which there may be several groups of linked loci.
- pwld - Pairwise linkage disequilibrium measures.
- rclogit - Conditional logistic regression with robust standard errors.
- snp2hap - Infer haplotypes of 2-locus SNP markers.
- tdt - Classical TDT test.
- trios - Tabulate genotypes of parent-offspring trios.

Programs by Adrian Mander

- gipf - Graphical representation of log-linear models.
- hapipf - Haplotype frequency estimation using EM algorithm and log-linear modelling.
- pedread - Read pedigree data file (in pre-Makeped LINKAGE format), similar to ginsheet
- pedsumm - Summarises a pre-Makeped LINKAGE file.
- pedraw - Draws one pedigree in the graphics window
- plotmatrix - Produces LD heatmaps displaying graphically the strength of LD between markers.
- profhap - Calculates profile likelihood confidence intervals for results from hapipf
- swblock - A step-wise hapipf routine to identify the parsimonious model to describe the Haplotype block pattern.
- qhapipf - Analysis of quantitative traits using regression and log-linear modelling when phase is unknown.
- hapblock - attempts to find the edge of areas containing high LD within a set of loci

Other programs

By Mario Cleves

- gencc - Genetic case-control tests
- genhw - Hardy-Weinberg Equilibrium tests
- qtlsnp - A program for testng associations between SNPs an a quantitative trait.

By Catherine Saunders

- co_power - Power calculations for Case-only study designs.
- gei_matching -
- geipower - Power calculations for Gene-Environment interactions.
- ggipower - Power calculations for Gene-Gene interactions.
- tdt_geipower - Power calculations for Gene-Environment interactions via TDT analysis.
- tdt_ggipower - Power calculations for Gene-Gene interactions via TDT analysis.

By Neil Shephard

- genass - Performs a number of statistical tests on your genotypic data and collates the results into a Stata formatted data set for browsing.

Programs for GWAS

By Chuck Huber

- phasein/phaseout – input/output with PHASE
- haploviewin/haploviewout – input/output with HAPLOVIEW

By Jian'an Luan

- qc – genomic control using p values
- gwa – genomewide analysis using SNPTEST
- ...

By Jing Hua Zhao

- stata_snphwe – a Stata plugin for exact test of Hardy-Weinberg equilibrium using genotype counts

III Miscellaneous topics

Topics

- Meta-analysis
- Risk prediction
- Instrumental variable method and structural equation modeling
- Gaussian graphical models and networks
- Extreme value modeling

Meta-analysis

- Some circulations within the GIANT consortium considered two studies with sample sizes 32000 and 8000 both with p values 1e-8, we have a combined two-sided p value of 1.49e-14 but also yields p=4.89e-8 with p_1=1e-4 and p_2=1e-5 (weighted z-score method from *metap* in gap).
- In general, it statistically combines data from multiple studies in the consortium to learn about association (level of significance) and factors related to variations in its magnitude (effect size). We have test of significance = size of effect x size of study, e.g., $\chi_1^2 = r^2N$ (Kramer & Rosenthal. Comprehensive Clinical Psychology 3-15, Elsevier 1998)

Combining independent tests

- Fisher's method
- One can use truncated p values
- Stouffer's method is based on normal approximation.
- The R implementation is straightforward with sum(-2 * log(pvalues))) and sum(qnorm(1-pvalues)) / sqrt(k)).

$$\chi^2_{2k} = -2\ln P_i \quad i = 1,...,k,$$

$$z = 1/\sqrt{K}\,\Sigma_{i=1}^{k}\Phi^{-1}(1-P_i)$$

- Fisher's method has limitations in
  - Giving equal weight to studies with different sizes
  - No test of heterogeneity
  - No point estimate to become more precise as K increases
- However, there is suggestion about bias regarding msSNP.

Regression models for meta-analysis

- Fixed effects model is unable to account for heterogeneity since deviations from θ_i and θ are assumed to be explained by random error.
- Random effects model. It is assumed that each study has its own effect distribution against a common distribution.
- The popular DerSimonian-Laird (DL, moment) estimator equates the expectation of the heterogeneity statistic.
- We can include covariates in the model to make study-specific adjustments, i.e., meta-regression.
- Simple heterogeneity (SH) model uses GLS with strictly positive variance estimate.

$$\theta_i = \theta + \varepsilon_i, i = 1,...,k,$$

$$\varepsilon_i \sim N(0, \sigma_i^2)$$

$$\theta_i = \theta + b_i + \varepsilon_i, i = 1,...,k,$$

$$b_i \sim N(0, \tau^2),\ \varepsilon_i \sim N(0, \sigma_i^2)$$

$$\hat{\tau} = \frac{\sum_{i=1}^{k} \sigma_i^{-2}(\theta_i - \hat{\theta})^2 - (k-1)}{\sum_{i=1}^{k} \sigma_i^{-2} - \sum_{i=1}^{k} \sigma_i^{-4} \Big/ \sum_{i=1}^{k} \sigma_i^{-2}}$$

$$\theta_i = \theta + \theta_1 z_i + b_i + \varepsilon_i, i = 1,...,k,$$

$$b_i \sim N(0, \tau^2),\ \varepsilon_i \sim N(0, \sigma_i^2)$$

$$Var(\hat{\theta}_i) = \tau^2 + \sigma^2 = \tau^2(r_i + 1)$$

$$E(\hat{\theta}) = X\theta, Var(\theta) = \tau^2 V$$

Measure of Heterogeneity

- Cochran's Q, $Q = \sum_{i=1}^{k} (\theta_i - \bar{\theta})^2 / \sigma_i^2$, can be referred to a chi-squared distribution with k-1 degrees of freedom.
- I^2, defined as 100%(Q-df)/Q, which expresses the percentage of between-study variability that is attributable to heterogeneity rather than chance. Thresholds of 20%, 50%, and 75% are suggested to have low, moderate and high heterogeneity (Higgins *et al*. *BMJ* 2003; 327:57-60).
- It has been suggested that $cQ \sim x^2(v)$ with Q being heterogeneity chi-square, has excellent property (Bohning et al. 2008).

Implementations

- SAS has no built-in procedure for meta-analysis but can customarily done via PROCs GLM (fixed effects/inverse variance) and more often MIXED as well as macros.
- Stata has a comprehensive collection of meta-analysis, notably metan.
- R hosts several package at CRAN (e.g., meta, rmeta) .
- S-PLUS has user-written packages, e.g., hblm.
- Others such as HLM, MLwiN, WinBUGS.
- Customized programs

Useful URLs

- CAMAN (Computer Assisted Analysis of Mixtures)
  http://www.charite.de/biometrie/schlattmann/book/
- improved.ci (function for the improved confidence interval using DL method)
  http://www.statistik.tu-dortmund.de/ma_book.html
- hblm (Hierarchical Bayes Linear Model Programs)
  ftp://ftp.research.att.com/dist/bayes-meta/
- CAMAP (Computer-Assisted Meta-Analysis with the Profile Likelihood)
  http://www.personal.reading.ac.uk/~sns05dab/Software.html

Fixed-effects meta-analysis

```
data test;
        input studyid lor est;
        col=_n_; row=_n_;
        value=est;
cards;
... data for 15 studies ...
run;
proc mixed method = ml data=test;
       class studyid;
        model lor = / s cl;
        repeated / group = studyid;
        parms / parmsdata=test eqcons=1 to 15;
run;
```

Random-effects meta-analysis

```
proc mixed data=test covtest;
      class studyid;
      model lor = / s cl outp=predp outpm=predm;
      repeated diag / r;
      random studyid / g gdata = test s v;
      ods output CovParms=cp G=G R=R V=V
                  SolutionF=SF SolutionR=SR;
run;
data predp;
      set predp; pvalue=probnorm(resid/stderrpred);
run;
data predm;
      set predm;pvalue=probnorm(resid/stderrpred);
run;
```

Stata

```
use meta5
list in 1/5
metan b se, by(snp) fixedi nograph
```

WinBUGS

```
model
{
  for (i in 1:r)
  {
    y[i] ~ dnorm(psi[i],w[i])
    psi[i] ~ dnorm(theta,t)
  }
  theta ~ dnorm(0,1.0E-4)
  t ~ dgamma(0.001,0.001)
  tausq <- 1/t
}

list(y = c(0.864, 0.646, 0.272, 0.916, 0.867, 0.819, 0.809, 1.212,
     -0.273), w = c(4.40, 9.89, 16.81, 8.38, 8.15, 10.36, 10.79, 4.40,
    15.95), r = 9)
list(theta = 0, t = 1, psi = c(0,0,0,0,0,0,0,0,0))
```

R/meta, R/rmeta, R/CAMAN

```
library(CAMAN)
data(aspirin)
aspirin
mix <- mixalg(obs="logrr", var.lnOR="var", data=aspirin)
library(rmeta)
attach(aspirin)
annotate <- cbind(name,year)
metaplot(logrr,se,labels=annotate)
library(meta)
mg <- metagen(logrr,se)
plot(mg)
funnel(mg)
metabias(mg, method=“linreg”)
```

R/meta and R/metafor with by

```
library(foreign)
setwd(".")
meta5 <- read.dta("meta5.dta")
attach(meta5)
library(meta)
s <- by(meta5,snp,function(x) metagen(b,se,data=x))
names(s)
names(s$rs998663)
library(metafor)
ss <- by(meta5,snp,function(x) rma(b,se,data=x))
names(ss$rs998663)
# Forest, Funnel, Radial and Residual plots
plot(ss$rs998663)
```

Customized programs

- META
- METAL
- MetABEL
- R/snpMatrix

A cautionary note

- In a meta-analysis, we compute effect size for each study and combine them but not combine summary data and compute an effects size for the combined data.
- This allows for a check of consistence regarding effect sizes across studies and minimizes the potential confounders.
- If we were to pool data across studies and then compute the effect size from the pooled data, we may get the wrong answer, due to Simpon's paradox.

See Chapter 13 of Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis.* Wiley 2009

Extensions: multivariate Meta-Analysis

- Background
  - A gene-based association testing (Neale & Sham) not dissimilar to the usual Fisher p value method
  - Multilocus scan statistics (Hoh & Ott) not taking off
  - Bayesian meta-analysis is more involved and the formulation via summary data as in Verzilli *et al*. is not not necessarily used.
  - P values adjusted for correlated tests (p_ACT, Conneely & Boehnke) addresses the following question: What is the minimum p value and more importantly given it is obtained what are the significant levels for all others?
- Problems
  - Covariance of association tests can be poorly estimated given multicollinearity between SNPs at a region/gene.

Statistical models

- The data typically involve *b*, *SE* from linear regression of nearby SNPs to allow for fixed- and random effects modeling and assessment of statistical significance.
- It is not obvious how to infer covariance matrix involving these *b*'s. However, we can work around with respect to pair-wise correlations (*r*).
- For linear regression, it is known that *r* and *t* (=*b*/*SE*) is related via a simple expression $r^2 = t^2/(n-2+t^2)$.
- The covariance between pair-wise correlation has the following form.

Covariance between pairs of correlations

$$Cov(r_{st}, r_{ut}) = [0.5\rho_{st}\rho_{ut}(\rho_{su}^2 + \rho_{st}^2 + \rho_{tu}^2 - 1) + \rho_{su}(1 - \rho_{st}^2 - \rho_{ut}^2)]/n$$

$$Cov(r_1, r_2) = [\rho_1\rho_2\rho_{12}^2 + (\rho_1^2 + \rho_2^2 - 1)(\rho_1\rho_2 - 2\rho_{12})]/2n$$

$$\text{or } (1 - \rho^2)^2/n \text{ with } \rho_1 = \rho_2 \equiv \rho \text{ and } \rho_{12} = 1$$

Elston RC (1975). On the correlation between correlations. Biometrika 62:133-40

Combination of SNPs via GLS

- The results of k independent studies, each with p correlations, can be expressed as the concatenation of the vectors of all available correlations. The large sample variance-covariance matrix is then block diagonal. The estimation of the pooled correlation matrix can then be done via weighting or via a generalized least squares (GLS) framework.
- A test of homogeneity of correlation matrices among studies can be performed (Becker 1992). We can accommodate the heterogeneity via a random effects model such that population correction for specific study is a result of the population correlation and study specific factor.
- The implementation (e.g., in R) accounts for variable number of SNPs from each study (Verzilli et al. 2008).

p_ACT and p_ACT_meta

- p_ACT is based on multivariate normal (MVN) assumption originally for sample with individual genotypes but recently extended to results from consortium meta-analysis.
- The basic idea with p_ACT_meta is to find the minimum p value from the collection of correlated SNPs and obtain subsequent p values based on MVN conditional distributions (Holm's procedure) using R/mvtnorm.
- It uses a James-Stein shrinkage estimate as implemented in R/corpcor. A description of mvtnorm appears in *The R Journal*.
- However, the omnibus approach noted earlier is appealing.

Summary

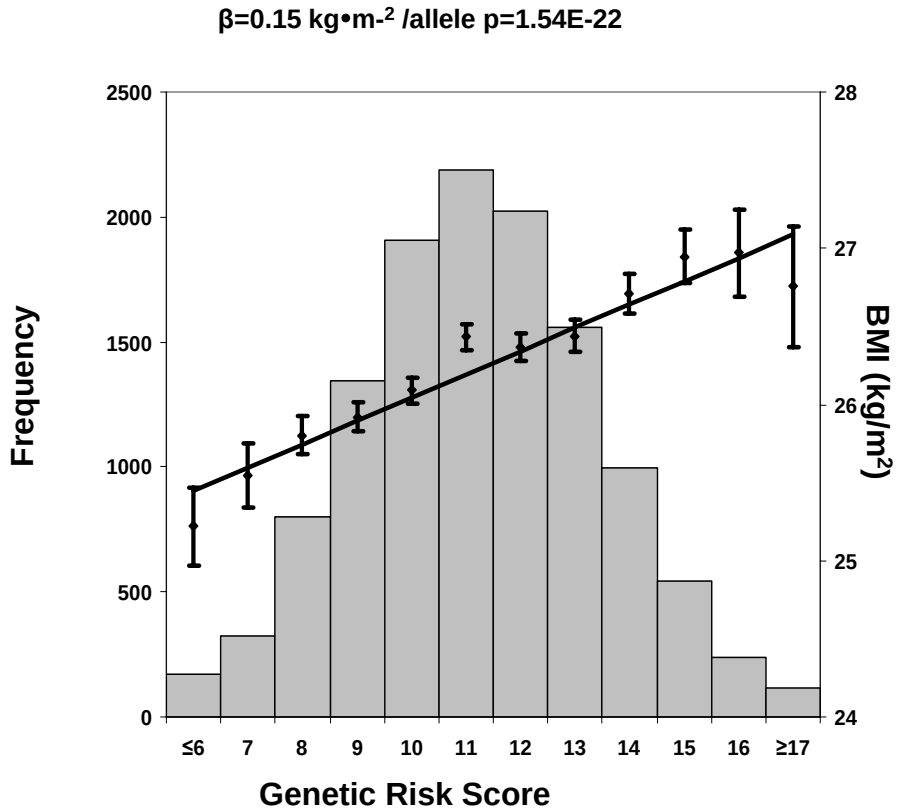
- It is far from a comprehensive overview but offers some flavour of the kind of thinking and practice.
- Evidence synthesis with conscious recognition of heterogeneity is in the heart of meta-analysis.
- Fixed effects analysis is restricted to data of the type found in the studies included, but random effects model generalizes to all studies of the type from which our studies were drawn. Results from both models together with SH model are highly recommended.
- We have omitted the graphical aspects, e.g., Bax *et al.* *AJE* 2009; 169:249-55. An Excel macro is available from http://www.mix-for-meta-analysis.info/index.html

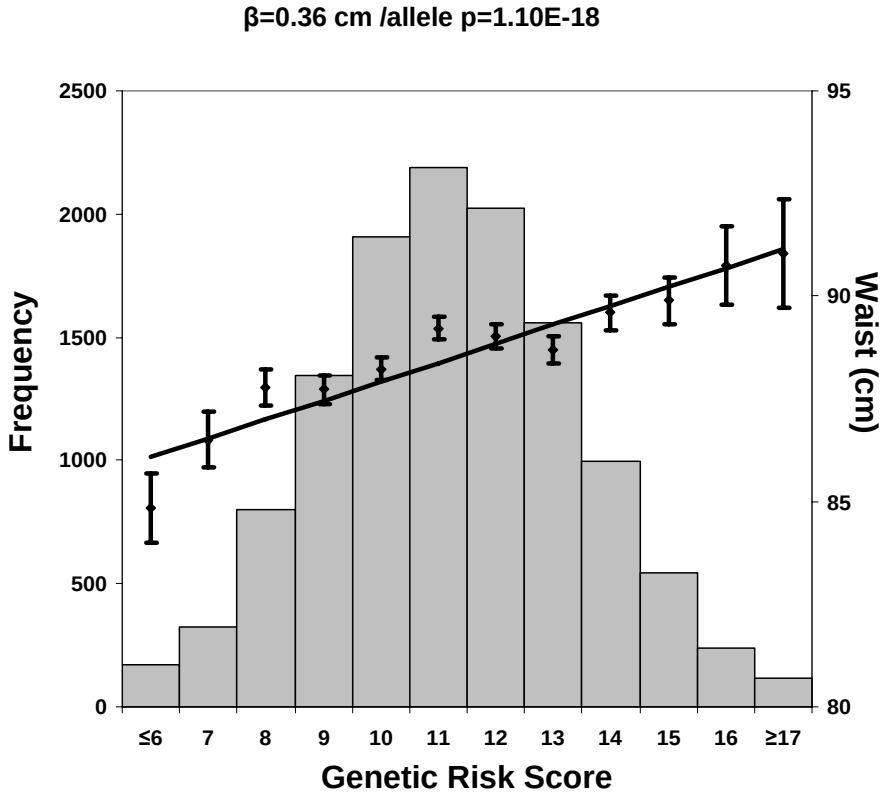
Risk prediction

- A set of SNPs can be used in a logistic regression model to predict if an individual is a case or control based on a cut-off probability. An optimal cut-off can be facilitated through receiver operating characteristics (ROC) curve. The ability to classify individuals correctly is measured by area under the ROC curve (AUC, e.g. ~0.5, 0.7-0.8, 0.8-1 for no, acceptable, excellent discrimination).
- Examples: prostate cancer, obesity, HDL/TG/LDL.
- A testing example

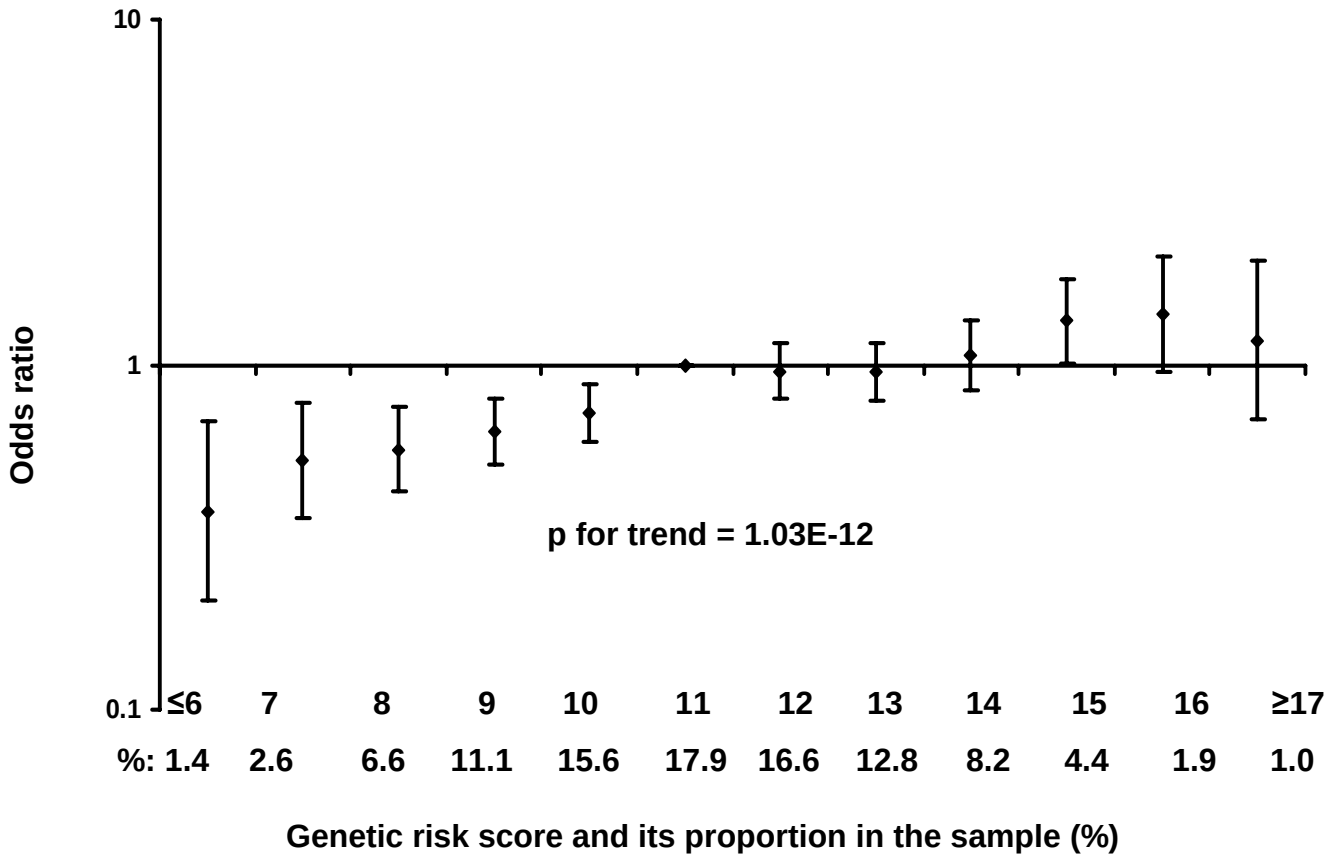
```
library(verification)
obs<- round(runif(100))
pred<- runif(100)
A<- verify(obs, pred, frcst.type = "prob", obs.type = "binary")
roc.plot(A, main = "Test", binormal = TRUE, plot = "both")
roc.plot(A, threshold=seq(0.1,0.9, 0.1), CI=TRUE, alpha=0.1)
roc.plot(obs,pred,xlab='1-specificity',ylab='sensitivity',cex=2)
AUC <- roc.area(obs,pred)$A
```

Risk score and BMI in EPIC-Norfolk

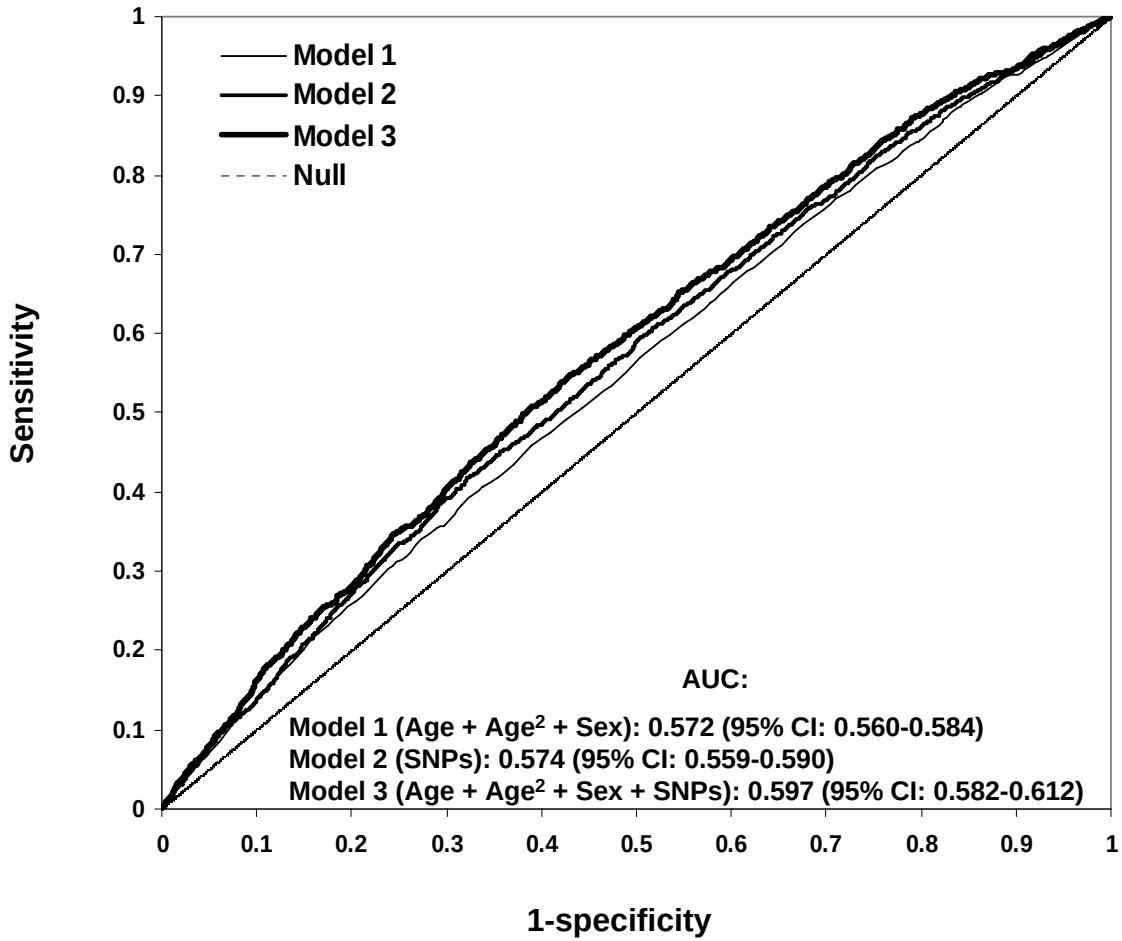




Risk score and obesity/overweight



ROC curve and AUC

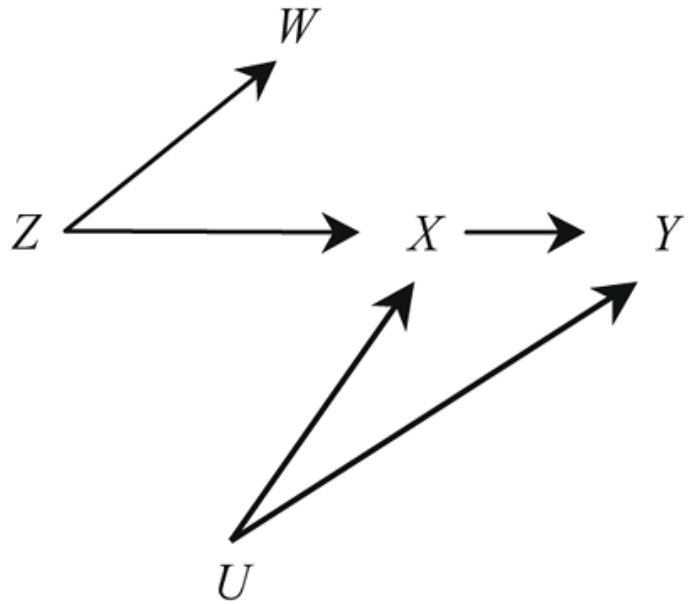


Instrumental variable (IV) estimation

- It is a method for estimating regression Y=(Z X)b+e parameters b when X are measured with error, W=X+U, and possibly when a second or biased but independent measurement (T) is available. Given cov(T,e) = cov(T,U)=0, cov(T,X)<>0, b=cov(T,Y)/cov(T,W).
- More formally, 1. T is uncorrelated with X; 2. T is independent of the measurement error U=W-X in the surrogate W; 3. (W,T) is a surrogate for X so that E(Y|Z,X,W,T)=E(Y|Z,X).
- See Fuller WA. Measurement Error Models. Wiley 1987; Greene WH. Econometric Analysis, 5e. Prentice Hall 2003; Carroll et al. Measurement Error in Nonlinear Models-A Modern Perspective, 2e. CRC 2006; Gelman A, J Hill. Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press 2007

IV in simple terms

In an observational study, U represents unmeasured confounders of the X–Y association. In a randomized trial, U represents variables that affect adherence to treatment assignment and thus influence received treatment X. Z is called an instrumental variable (or instrument) for estimating the effect of X on Y.



Rothman KJ, Greenland S, Lash TL. Modern Epidemiology, 3e, Lippincott Williams & Wilkins 2008

a. Z affects X (i.e., Z is an ancestor of X). b. Z affects the outcome Y only through X (i.e., all directed paths from Z to Y pass through X). c. Z and Y share no common causes.

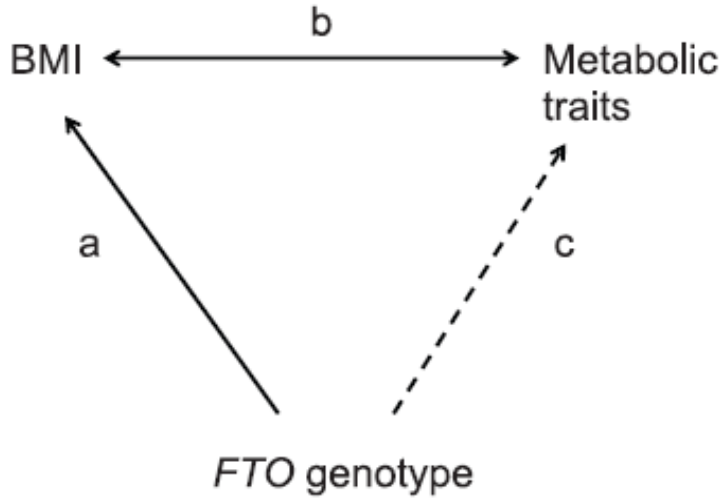
Instrumental-variables regression (IVLS)

- We can generalize the model as $Y_{nx1}=X_{nxp}\beta_{px1}+\delta$, IVLS or two-stage least squares (2SLS) requires that (i) X_{nxp} and Z_{nxq} with $n>q\geq p$. (ii) $Z'X$ and $Z'Z$ have full rank, p and q respectively. (iii) $Y=X\beta+\delta$. (iv) The δ_i are i.i.d. with mean 0 and variance σ^2. (v) Z is exogeneous, i.e., Z is independent of δ. The cases with $q>$, $=$, and $<$ p are called over-, just-, and under- identified, respectively.
- Solution to the system proceeds by multiplying Z on the Y-X model and rescaling by variance such that $(Z'Z)^{-1/2}Z'Y=(Z'Z)^{-1/2}Z'X\beta+\eta$, where $\eta=(Z'Z)^{-1/2}Z\delta$

Freeman DA. Statistical Models-Theory and Practice, Revised Edition. Cambridge University Press, 2009.

FTO genotype, BMI and metabolic traits

- There is epidemiological association between BMI and metabolic traits.
- There is association between *FTO* and BMI.
- The association between *FTO* genotype and metabolic traits would be mediated by BMI (c=axb).



- This is the so-called triangulation approach (Freathy et al. Diabetes 2008; 57:1419-26).

Direct and indirect effects

- We can lay out two equations

$$TG = \beta_0 + \beta_1 BMI + \beta_2 SNP + error$$
$$BMI = \gamma_0 + \gamma_1 SNP + error$$

- We can plug in the second equation into the first.

$$TG = \beta_0 + \beta_1 BMI + \beta_2 SNP + error$$
$$= \beta_0 + \beta_1(\gamma_0 + \gamma_1 SNP) + error$$
$$= (\beta_0 + \beta_1\gamma_0) + (\beta_1\gamma_1 + \beta_2)SNP + error$$

- We proceed with two steps:

1. We first regress TG on SNP.
2. We also regress BMI on SNP.

$$TG = \delta_0 + \delta_2 SNP + error$$
$$BMI = \gamma_0 + \gamma_2 SNP + error$$

- We then have the Wald estimate with $\beta_2 = 0$

$$\gamma_2 = \beta_1\gamma_1 + \beta_2$$
$$\beta_1 = (\delta_1 - \beta_2)/\gamma_1$$

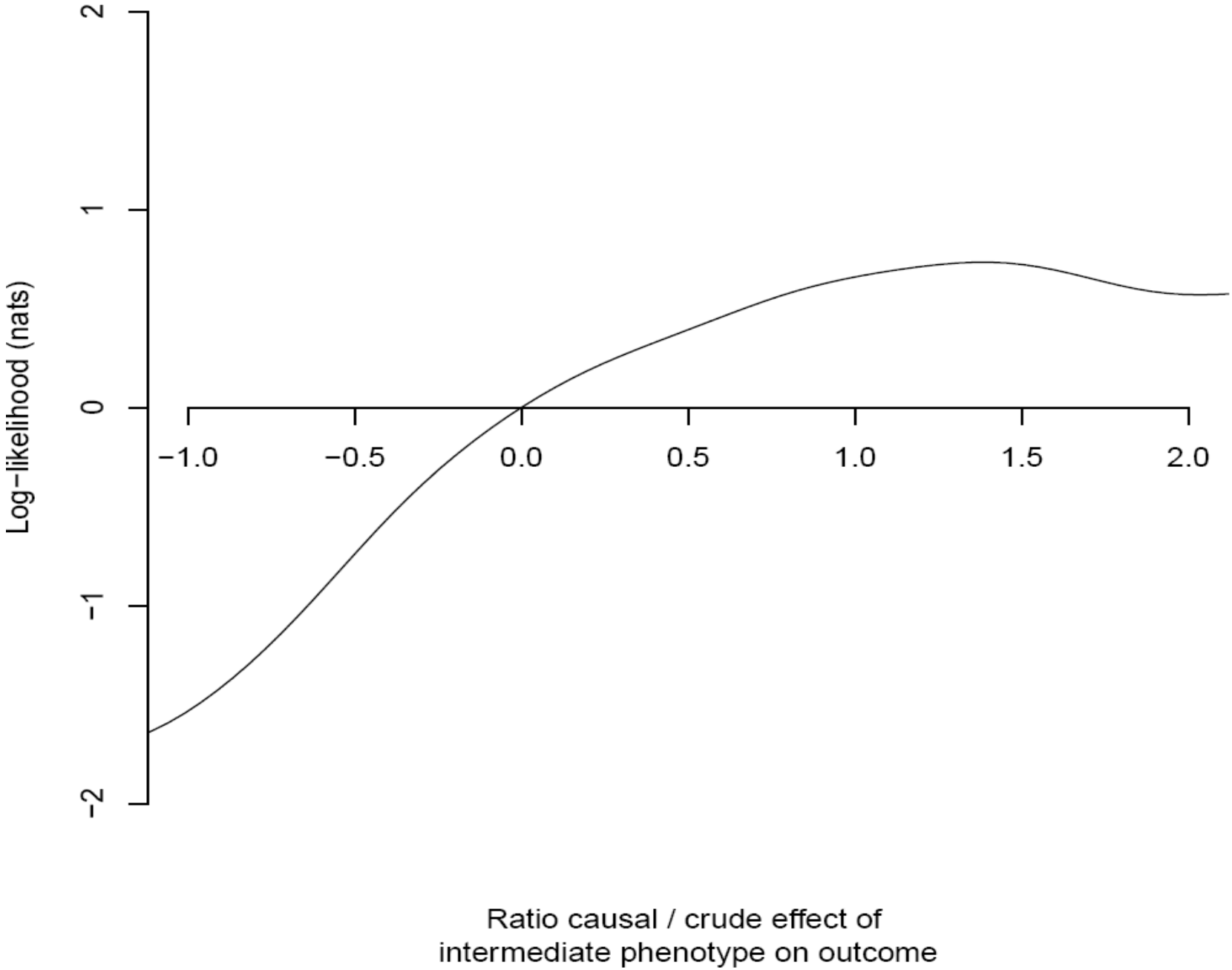
- A summary in our setting is Bochud et al. *IJE* 2008, 37:414-6

-1131T>C (rs662799), TG and CHD

1. –1131T>C, a regulatory variant in *APOA5,* is unrelated to several non-lipid risk factors or LDL cholesterol, and comparatively moderately related to HDL cholesterol and other major lipids. 2. –1131T>C is strongly related to TG concentration in a dose-dependent manner, with every C allele increasing TG by about as much as having type 2 diabetes mellitus. 3. in an analysis of 20 842 cases and 35 206 controls,–1131T>C is related to risk of CHD in an analogous dose-dependent manner, with about 18% higher risk per C allele. 4. in an analysis of 302 430 people, risk of CHD with genetically raised TG is concordant with risk of disease with equivalent differences in circulating TG itself. 5.–1131T>C is associated with higher VLDL concentration and smaller HDL particle size—pathways through which TG could affect risk of coronary heart disease. (*Lancet* 375:1634-9, 2010)

SLC2A9, urate levels and metabolic syndrome

- This example was reported recently by McKeigue et al. *Int J Epidemiol*. 2010; 39:907-18
- The data contains 583 individuals with sex, age and seven SNPs, one of which is non-synonymous and used as instrumental variable.
- The R package mediation only accepts data without missing values, so we used 493 individuals.
- The authors implemented a Bayesian logistic models and have applied JAGS and have argued in favor of this model over probit model.



Parameter θ with values 1 vs 0 yields lod score of 2.24

Issues with IV

- No suitable genetic variant
- Unreliable gene association
- Population stratification
- Linkage disequilibrium
- Pleiotropy
- Nonlinear association
- Weak instrument
- ...

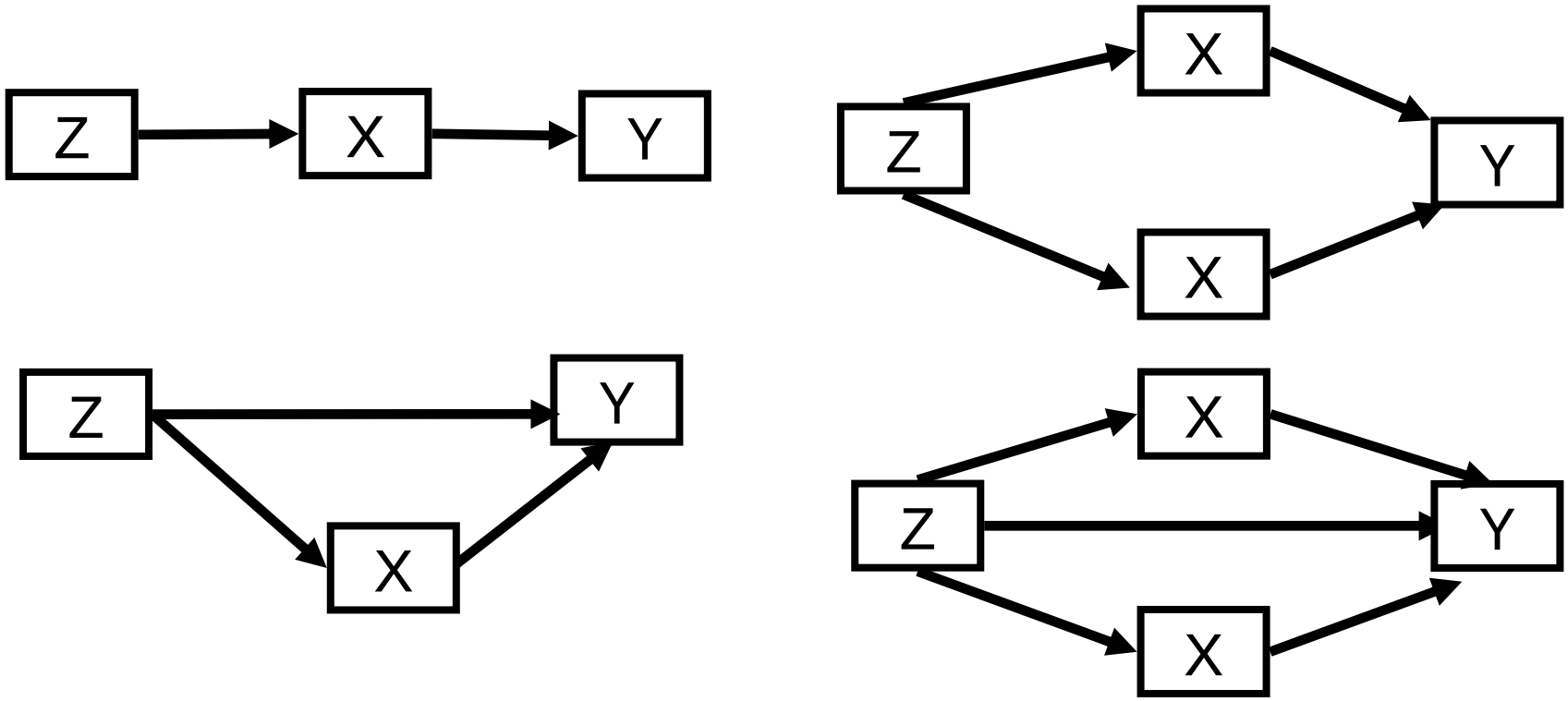
See Lawlor et al. (2007). *Stat Med* 27:1133-67; Didelez & Sheehan (2007). *Stat Meth Med Res* 16:309-30; Didelez et al. *Stat Sci* 2010. Pare & Anand (2010) Lancet 375:1584-5

Warnings against categorical data

- Three models are involved with binary outcome (y), mediator (M), and predictor (X): $y=i_1+cX+e_1$, $y=i_2+c'X+bM+e_2$, $M=i_3+aX+e_3$ such that when (c-c') is is employed, its standard error becomes more complicated than ordinary linear regression. It is often to set the residual variance in logistic regression to be $\pi^2/3$ and probit regression to 1. The mathematical tractability of multivariate probit distribution makes it appealing in modeling categorical variable with Mplus.
- A single formula to standardize according to estimates from $y=i_1+cX+e_1$.

- See MacKinnon DP. Introduction to Mediation Analysis. Lawrence Erlbaum Associates, 2008

Mediation analysis



Scenarios of mediation: complete (upper left), partial (lower left), complete (upper right) and partial (lower right) with two mediators

The *SLC2A9* example

```
library(foreign)
snp <- read.dta("mediate.dta")
library(mediation)
B=lm(x~nsg+sex+age+rs3766404+rs6677604+rs1329428+rs11582939+CFHR3R1del+rs7517126,data=snp)
c=glm(y~x+sex+age+nsg+rs3766404+rs6677604+rs1329428+rs11582939+CFHR3R1del+rs7517126,
    family=binomial(link="logit"),data=snp)
logitm <- mediate(b, c, sims=10000, treat="nsg",
    mediator="x")
summary(logitm)
```

We obtain comparable results with probit link.

Results

Quasi-Bayesian Confidence Intervals

Mediation Effect: -0.006834 95% CI -0.022355 0.002811

Direct Effect: -0.1205 95% CI -0.19597 -0.02652

Total Effect: -0.1273 95% CI -0.20195 -0.03293

Proportion of Total Effect via Mediation: 0.04556 95% CI 0.02904 0.15595

Structural equation modeling

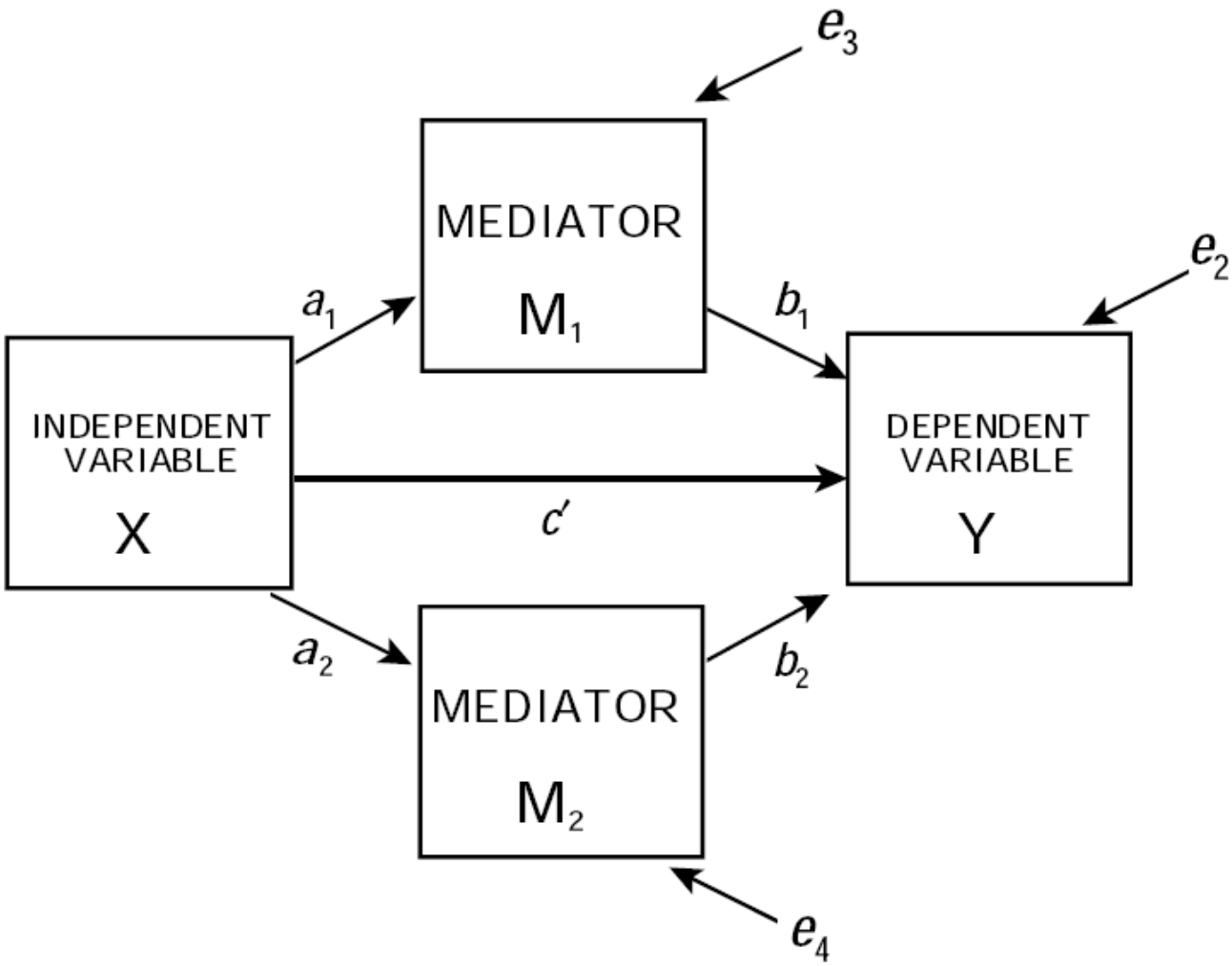
- Several examples seen in recent GWAS literature can be modeled via path analysis or put in this framework.
- It is typically confirmatory based on model-fitting.
- It has been a rather useful device to study causal relationship.
- It is natural to study change using longitudinal data.
- sem package in R is a very good initiative, but it is often necessary to resort to other systems such as EQS, AMOS, M*plus,* e.g., the inter-relationship between anthropometric measurements using M*plus*.
- A critique is that SEM relies on conditional independence assumptions with IV being as a special case, so that the assumptions required for causal effects are difficult to satisfy. It is helpful to examine equivalent models.

M*plus* code

```
Title:
 snp1: rs1121980 from FTO
 snp2: rs17782313 from MC4R
 zlbmi : BMI
 zlwst : waist
 zltg : Triglycerides
 zsys : SBP
 zdia : DBP
Data:
 File is effectsize.dat ;
Variable:
 Names are snp1 snp2 zlbmi
zlwst zltg zsys zdia;
 Missing are all (-9999) ;
 Usevariables are snp1 zlbmi zltg;
```

```
Model:
 zltg on zlbmi;
 zlbmi on snp1;
 zltg on snp1;
Model indirect:
 zltg ind snp1;
Output:
 Standardized;
```

Two mediator model

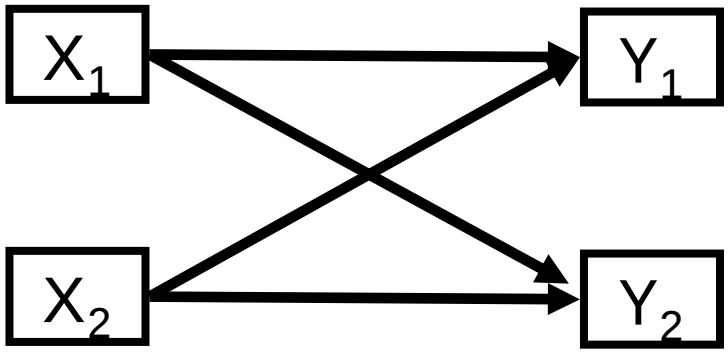


M*plus* for two mediator model

```
TITLE: two mediator example;
DATA:
NOBS = 400;
NGROUPS = 1;
FILE IS mediate2.dat
VARIABLE:
NAMES ARE ID x m1 m2 y;
USEVARIABLES ARE x m1 m2 y;
ANALYSIS:
TYPE IS GENERAL;
ESTIMATOR IS ML;
ITERATIONS = 1000;
CONVERGENCE = 0.000001;
```

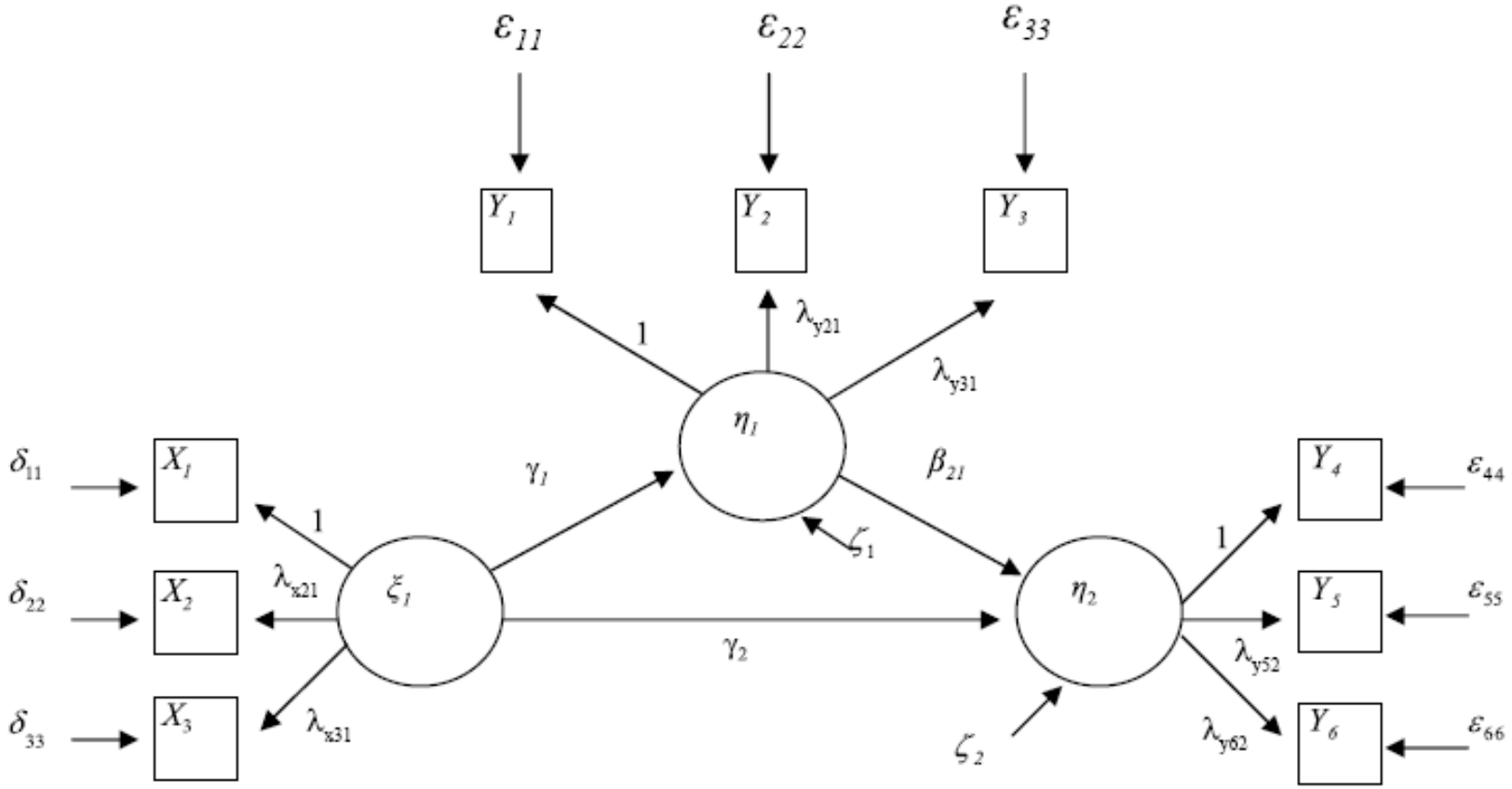
```
MODEL:
y ON m1 m2 x;
m1 ON x;
m2 ON x;
m1 with m2;
MODEL INDIRECT;
y IND x;
```

More complicated scenarios



When Y_1 becomes X_2 and X_2 becomes Y_2, the cross-lagged model can be used to study reverse causation, especially with longitudinal data. It becomes clear that we will be most comfortable with the SEM framework, as is also illustrated with the following slide.

Latent mediator model



Bayesian networks

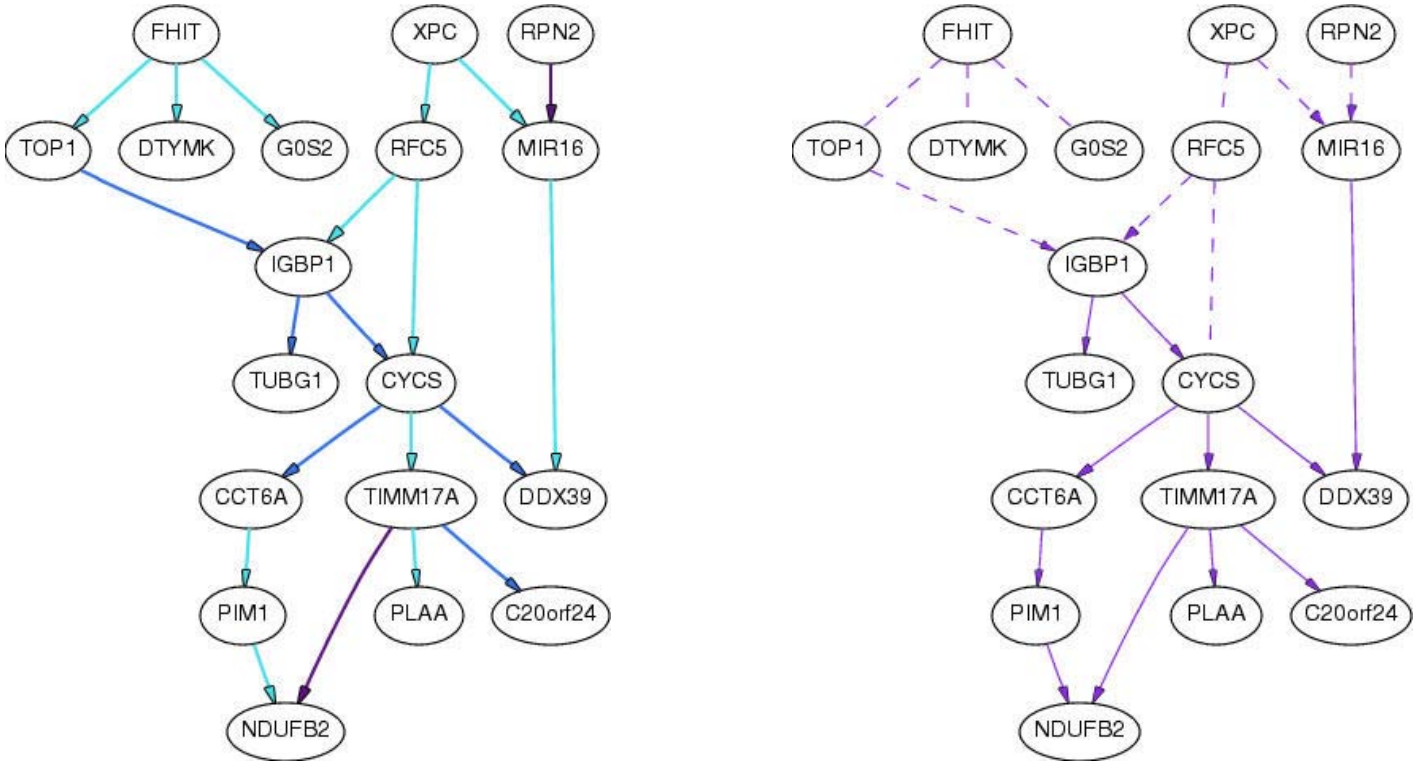
- Rule-based systems with certainty factors have serious limitations as a method for knowledge representation and reasoning under uncertainty, and attention towards a probabilistic interpretation of certainty factors leads to Bayesian networks.
- It can be described briefly as an acyclic directed graph (DAG) which defines a factorization of a joint probability distribution over the variables represented by the nodes of the DAG.
- The process of construction involves identification of the relevant variables and their causal relations, which leads to DAG specified in terms of a set of conditional probabilities.

Example-GAW15 Problem 1 data

- It was a published data (Morley *et al Nature* 2004, **430:** 743-74) on baseline expression levels of 8793 genes in immortalised B cells from 194 individuals in 14 CEPH pedigrees, shown to have linkage and association and evidence of substantial individual variations. In particular, correlation was examined on expression levels of 31 genes and 25 target genes corresponding to two master regulatory regions. We apply Bayesian network analysis to gain further insight into these findings.
- If the expression level of a given gene is regulated by certain proteins then it should be a function of the active levels of these proteins. Due to biological variability and measurement errors, the function would be stochastic rather than deterministic.
- Expression levels of genes are proxies for the activity level of the proteins they encode, although there are numerous examples where activation or silencing of a regulator is carried out by post-transcriptional protein modifications

Methods

- Gene expression levels as continuous variables were assumed to follow a multivariate normal distribution, and consistent with a Bayesian network with linear Gaussian conditional densities. The prior of this network is characterised by a prior network reflecting our belief in the joint distribution of the variables in question, and equivalent sample size (ESS) effectively behaving as if it was calculated from a “prior” data set of that size. For instance, without a priori knowledge of the regulatory network, the prior network could be one where all expression levels are independent in order to avoid explicitly biasing the learning procedure to a particular edge.
- The learning procedure starts with a training set and evaluates networks according to an asymptotically consistent scoring function that is obtained through the Bayesian framework. The so-called causal structure assumes that dependencies between variables are due to causal relationships between variables in the model.



Left. Importance of the dependencies. **Right**. Solid arc has direct causal influence (direct meaning that causal influence is not mediated by any other variable that is included in the study). Dashed arc indicates there are two possibilities, but we do not know which holds. Dashed line without any arrow heads indicates there is a dependency but we do not know the reciprocal dependence. From Zhao *et al. BMC Proc* 1:S52, 2007

Highlights of the analysis

- The series of papers on these data stress the importance of Intermediate phenotypes. Without a priori biological hypothesis, it serves as an exploratory tool for subsequent confirmatory analysis.
- This particular analysis highlights the potential usefulness of pathway analysis. An apparent limitation of this work, though not uncommon in gene-expression studies, is the relatively small sample size used. To fully elucidate the biological pathways involved may be difficult, as for instance CYCS is involved in a number of pathways.
- Statistical robustness and biological interpretability remain as the two main challenges for Bayesian network analyses, to which replication, bootstrap and benchmarking have been proposed.
- Our inference of gene networks also exploits the covariance structure of the data, like structural equation modelling, but is exploratory or hypothesis-generating rather than confirmatory or hypothesis-driven. A number of other software systems are of interest.

A Gaussian graphical model

We models measurements in EPIC-Norfolk data. The full, sub and final models give deviances of 0, 86.5, and 3.5, corresponding to df=0,1,1, respectively.

```
library(ggm)
all <- read.dta("ggm.dta")
all <- subset(all,!is.na(height+hip))
cor(all)
grm <-
    UG(~weight*bmi+weight*waist+weight*hip+waist*hip+waist
    *bmi+hip*bmi)
fit <-fitConGraph(grm,cor(all),n=2413)
grm <-
    UG(~weight*bmi+weight*waist+weight*hip+waist*bmi+hip*
    bmi)
fit <-fitConGraph(grm,cor(all),n=2413)
grm <- UG(~bmi*waist+bmi*hip)
fit <-fitConGraph(grm,cor(all),n=2413)
```

Extreme value theory

- It is concerned with questions related to extreme values in sequences of random variables and in stochastic processes, e.g. $M_n = \max(X_1,\ldots,X_n)$. An established results state that $P((M_n - b_n)/a_n) \to H(x)$ which are of three types and can be combined into a single Generalized Extreme Value (GEV) distribution.
- The distribution of X conditionally on some high threshold often has a limit which follows Generalized Pareto Distribution (GPD).
- An associate model considers r largest order statistics.
- See Finkenstädt B, Rootzén H. Extreme Values in Finance, Telecommunications, and the Environment Chapman and Hall/CRC 2003 and also http://www.stat.unc.edu/postscript/rs/semstatrls.pdf

Annual maximal levels of River Nidd

The data can be used as follows,

```
library(evir)
qplot(nidd.annual)
data(nidd.annual)
nidd.gev <- gev(nidd.annual)
plot(nidd.gev)
meplot(nidd.annual)
shape(nidd.annual)
pfit <- gpd(nidd.annual, threshold=200)
plot(pfit)
quant(nidd.annual)
```

Summary

- We have covered a variety of topics ranging from meta-analysis to causal modelling, which is expected to be more familiar with more genetic variants being established.
- They are general since some topics are also quite familiar to researchers at other fields (e.g., psychology, social science, econometrics) where for instance structural equation modelling are routinely used.

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IV OpenMx and NCBI2R

Topics

- Heritability estimation
  - Background
  - Family data
  - Twin data
  - OpenMx
  - Summary
- Information retrieval with NCBI2R
- Further information

Definition of genetic heritability

- Genetic heritability is defined for a quantitative trait as the proportion of variation attributable to genetic factors, and extended to categorical traits through reference to a liability model. The value of the genetic heritability varies according to factors taken into account.
- Let phenotype P has mean μ and variance σ^2 from a linear model $P=a+d+c+e$ where a, d, c and e represent additive, dominance, common environment and individual specific environment, then genetic heritability in the narrow sense is σ_a^2/σ^2 in contrast to genetic heritability as σ_g^2/σ^2, which can include epistasis.

Hopper JL. Heritability. In Armitage, P. Colton T (Eds). Encyclopedia of Biostatistics, 2e, Wiley 2005.

Some clarifications

- For a binary trait, such as whether or not an individual has a disease, heritability is not the proportion of disease in the population attributable to or caused by, genetic factors.
- For a continuous trait, genetic heritability is not a measure of the proportion of an individual's score attributable to genetic factors. Heritability is not about cause *per se*, but about the causes of variation in a trait across a particular population.
- As heritability varies according to which factors are considered, there is no unique value of genetic heritability of a characteristic. It also varies from population to population. A poorly measured trait will apportion to measurement error leading to lower estimate of genetic heritability.

Heritability studies

- Family studies
- Adoption (rearing of a nonbiological child) studies
- Migrant studies – migrants carry a risk reflecting country of origin
- Twin study – differences between monozygotic and dizygotic twins can be attributed to genetic influence

The case of obesity

- BMI is often used as surrogate measurement, such that those with BMI >=25 and >=30 are considered as overweight and obesity.
- The heritability estimate of BMI had a range of 30-70%, and 50-90% from twin studies. Maes et al showed to be ~70% based on meta-analysis.

Peterson et al. ... (twin study)... *JAMA* 256:2958, 1986

Stunkard et al ... (adoption study) .... *New Eng J Med* 314:193-8, 1986

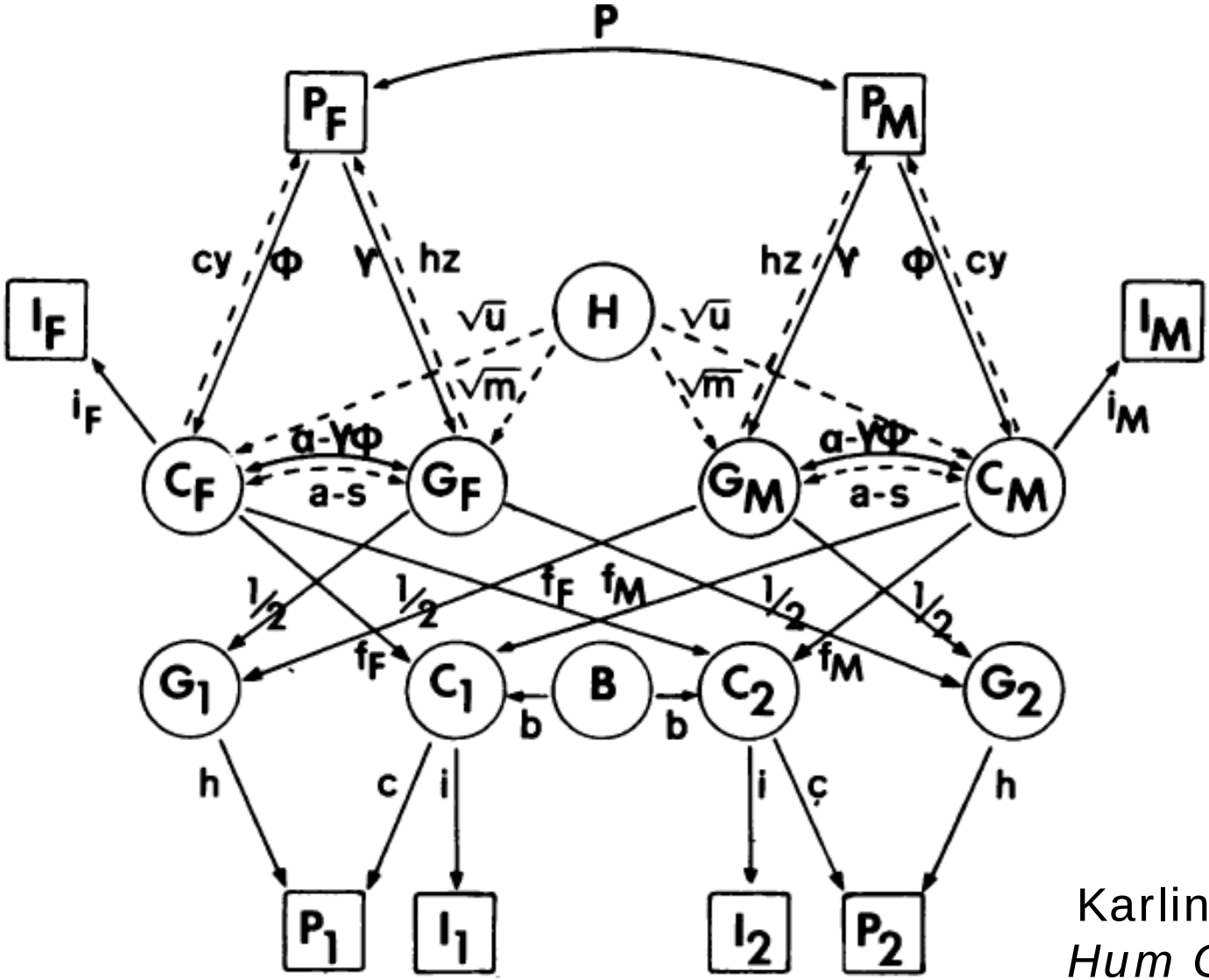
Maes et al. *Behav Genet* 27:325-51, 1997

Friedman JM. *Nat Med* 10:563-9, 2004

Path analysis of nuclear family data

- Recall that for the model $P=G+C+E$, can be re-expressed with path coefficients so that $P=hG+cC+E$ and to allow for intergenerational difference this can be written as $P=hzG+cyC+E$ for parents.
- We can also allow for correlation between parental phenotypes (homogamy) as with gene-environment correlations.
- We have the following path analysis model, noting that it uses the notion of “environmental indices” and sometime transformation of the phenotype for normality.

Mixed homogamy model



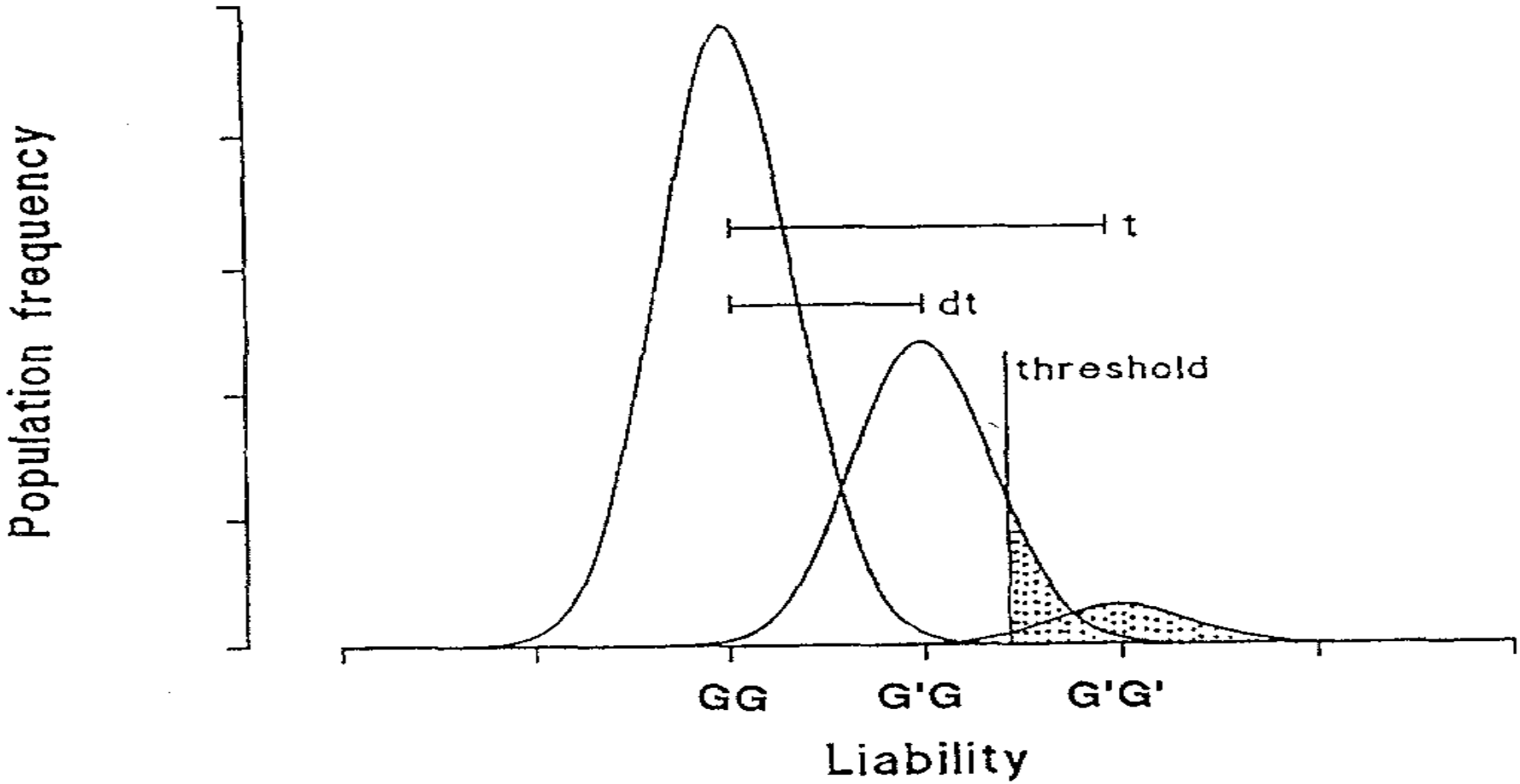
Karlin et al. *Am J Hum Genet* 1983; 35:695-732

h	Effect of child's genotype on child's phenotype
hz	Effect of parental genotype on parental phenotype
c	Effect of child's environment on child's phenotype
cy	Effect of parental environment on parental phenotype
p	"Primary" correlation between parental phenotypes due to phenotypic homogamy
m	Correlation between parental genotypes due to social homogamy
u	Correlation between parental environments due to social homogamy
f_F	Effect of father's environment on child's environment
f_M	Effect of mother's environment on child's environment
b	Effect of common sibship environment on child's environment
i	Effect of child's environment on child's index
i_F	Effect of father's environment on father's index
i_M	Effect of mother's environment on mother's index
γ	Correlation between parental genotype and parental phenotype
ϕ	Correlation between parental environment and parental phenotype
s	Correlation between adult's environment and spouse's genotype due to social homogamy
a	Total correlation between parental genotype and parental environment

Segregation analysis of family data

- When family data is available, we can examine major gene effect(s) together with collective loci with small and individually unmeasurable effects in a so-called mixed model (Morton NE, MacLean CJ. *Am J Hum Genet* 1974;26:489-503).
- It can also incorporate parameters for covariates such as age, sex and race.

Segregation analysis of NIDDM



Cook et al. *Diabetologia* 1994; 37:1231-40

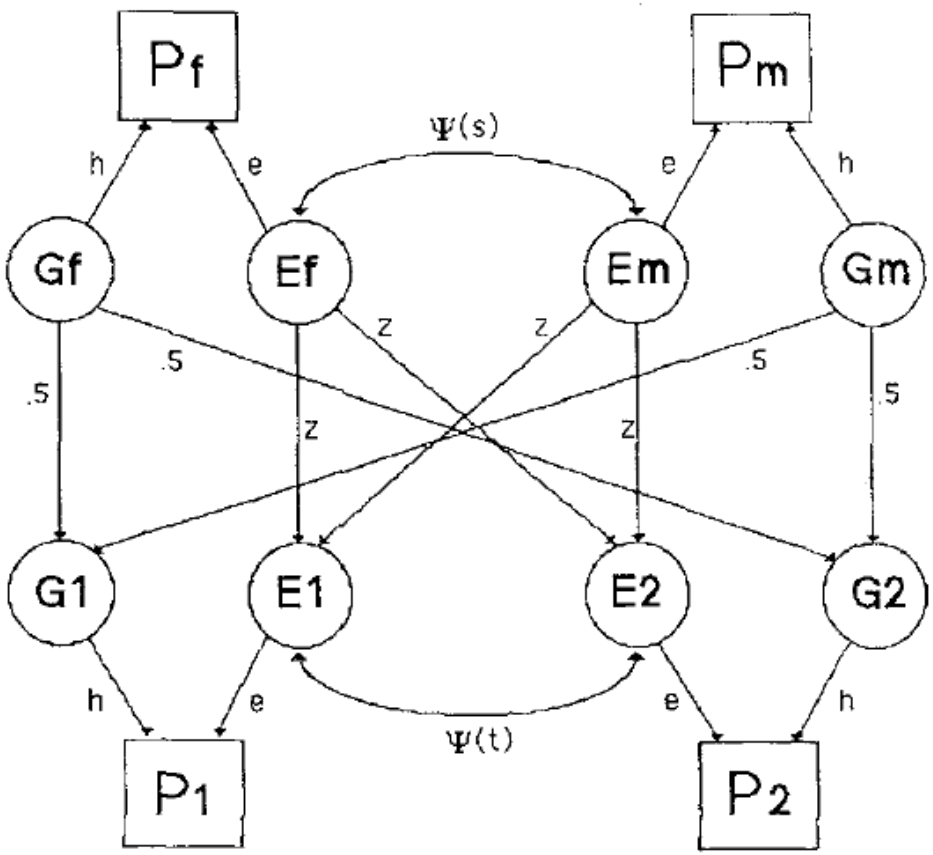
PATHMIX, ATRIBUTE and POINTER

- PATHMIX and ATRIBUTE provides path analysis of nuclear family data for both quantitative and binary traits.
- POINTER (Lalouel JM, Morton NE. *Hum Hered* 1981; 31:312-21) provides MLE of d - degree of dominance, which ranges between 0 for a recessive gene and 1 for a dominant; t - displacement between the two homozygotes of the major gene; q - gene frequency of allele leading to affection.
- Some attempt was made to include in R/CGR bundle.
- In addition, PAP and more recently JPAP has also implemented the mixed model of segregation and linkage.

Obesity in familial NIDDM

- The genetic model specified phenotype as the sum of independent effects attributed to the segregation of alleles at major loci, the transmission of polygenes, and random factors specific to the individual. The parameters were the total mean (g), the total standard deviation (a), the frequency of the allele determining high BMI at locus L (q_L), the dominance at locus L (d_L), the displacement at locus L (t_L), polygenic heritability (h^2), and parent-to-offspring transmission probabilities (t_1, t_2, and t_3 with values 1, 0.5, and 0 for Mendelian inheritance) for the three genotypes at one locus. Displacement is the difference, in within genotype SDs, between the means of two homozygotes. Dominance is the difference between the mean for heterozygotes and the mean for homozygotes, for low BMI relative to the displacement. The polygenic heritability is the proportion of the variance within major-locus genotypes, owing to polygenic inheritance.
- Hasstedt et al. *Am J Hum Genet* 1997; 61:668-77

Parent-offspring model with LISREL



- Boomsma et al. *Behav Genet* 1989; 19:123-41

SOLAR

- SOLAR (Sequential Oligogenci Linkage Analysis Routine, Almasy L, Blangero J. *Am J Hum Genet* 1998; 62:1198-211) uses likelihood ratio tests to evaluate heritability by comparing a purely polygenic model with a sporadic model in the case of testing heritability.
- In a polygenic model, *h2r* is the total additive genetic heritability. In a linkage model (with one or more locus specific elements) *h2q1* represents the heritability associated with the first locus, and *h2r* represents the residual genetic variance. In a oligogenic model, there may also be *h2q2, h2q3,* etc.
- Sung J, et al. *JCEM* 2009; 94:4946-52. reported a recent study of twins with adjusted (age, sex, age^2, age^2 x sex, total calorie intake, smoking and alcohol use) heritabilities for waist circumference (59%), glucose (59%), HDL (77%), TG (46%).

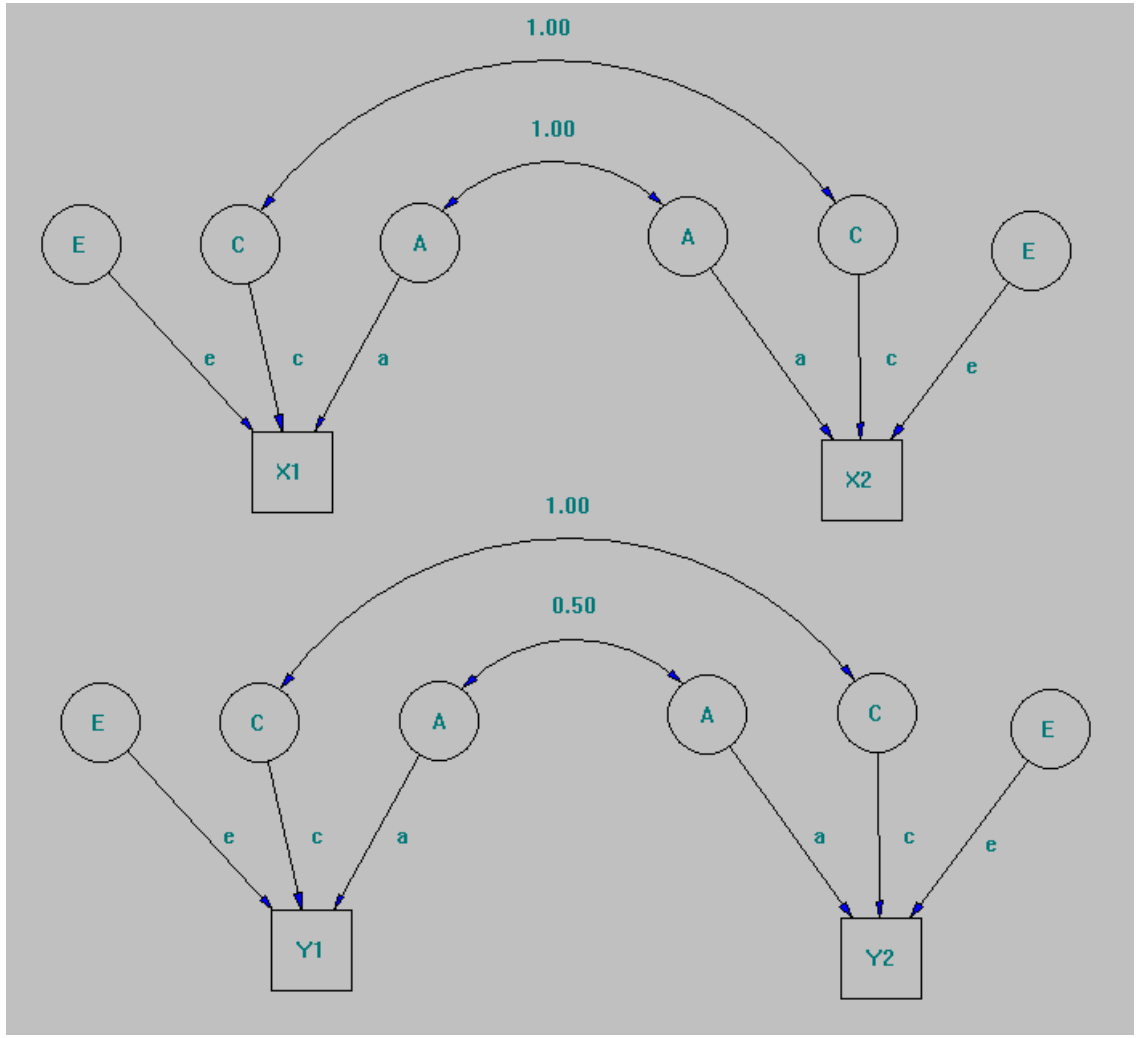
Twin ACE model

MZ

$$r_{MZ} = a^2 + c^2$$

DZ

$$r_{DZ} = 0.5a^2 + c^2$$



Recall that

- Expectations of sample correlation

  - $E(r) = \rho - \rho(1-\rho^2)/(2n)[1-(1-9\rho^2)/(4n)+\ldots]$
  - $V(r) = (1-\rho^2)^2/n[1+11\rho^2/(2n)+\ldots]$

Keeping ES. Introduction to Statistical Inference. Van Nostrand 1962; Dover 1995.

Simple estimation

- There we have mean/variance $a^2 = 2(r_{MZ} - r_{DZ})$

$$4\left[\left(1-r_{MZ}^2\right)^2 / n_{MZ} + \left(1-r_{DZ}^2\right)^2 / n_{DZ}\right]$$

- Similarly, $c^2 = 2r_{DZ} - r_{MZ}$

$$4\left(1-r_{MZ}^2\right)^2 / n_{MZ} + \left(1-r_{DZ}^2\right)^2 / n_{DZ}$$

$$e^2 = 1 - r_{MZ}$$

$$\left(1-r_{MZ}^2\right)^2 / n_{MZ}$$

- These can be simpler when there are equal numbers of DZ and MZ twins.

Maximum likelihood method

A notable implementation was twinan90 (Williams CJ, Christian JC, Norton JA Jr. (1992). *Comp Meth Prog Biomed* 38:(2-3):167-176)

```
library(gap)
fs <- file.path(.path.package("gap"),"tests/mzdz.dat")
mzdz <- matrix(scan(fs,skip=1),ncol=2,byrow=T)
mzdat <- mzdz[1:131,]
dzdat <- mzdz[132:206,]
twinan90(mzdat,dzdat,xlamb=2)
file.show(“mzdz.out”)
file.show(“mzdz.log”)
```

The estimation may be unstable.

A simulated twin data

```
library(mvtnorm)
mzm <- as.data.frame(rmvnorm(195, c(22.75,22.75),
matrix(2.66^2*c(1, 0.67, 0.67, 1), 2)))
dzm <- as.data.frame(rmvnorm(130, c(23.44,23.44),
matrix(2.75^2*c(1, 0.32, 0.32, 1), 2)))
names(mzm) <- names(dzm) <- names(mzw) <-
   names(dzw) <- c("bmi1","bmi2")
```

Summary statistics

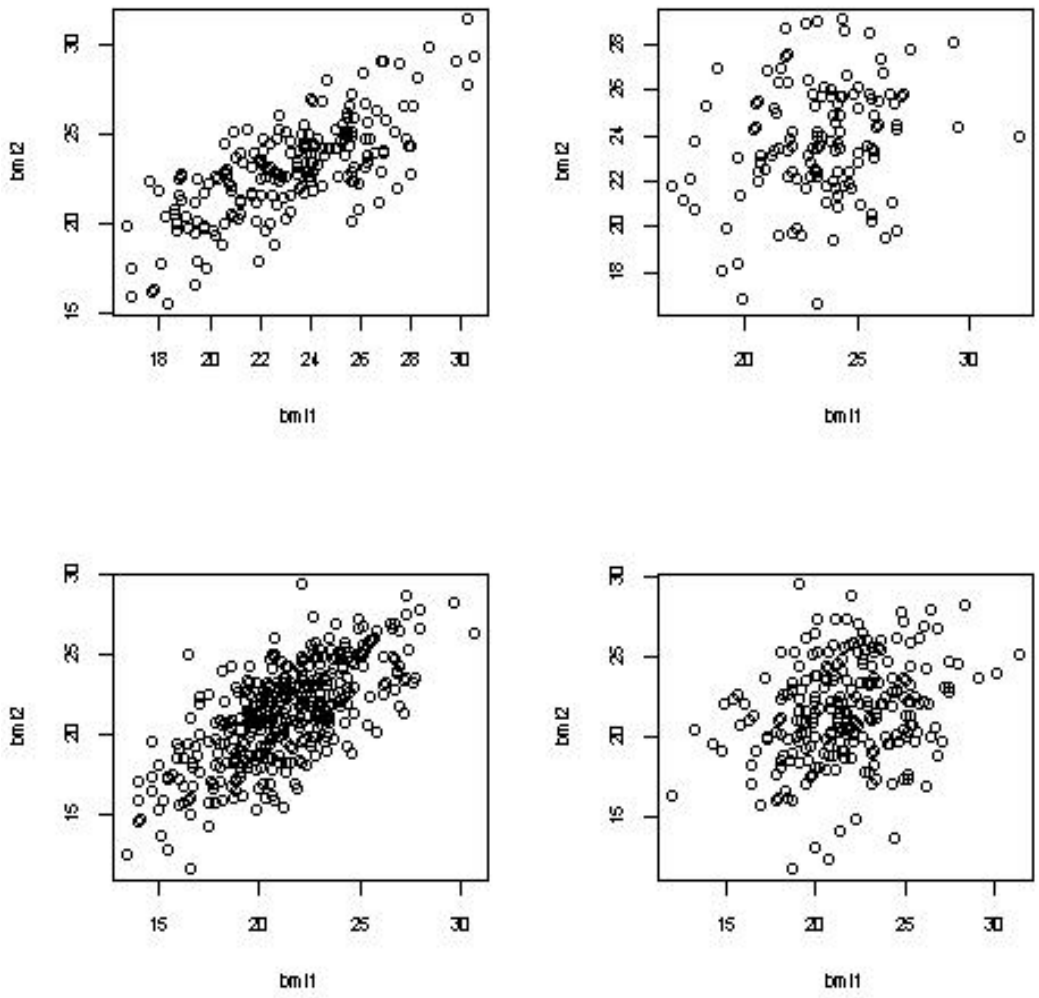
```
apply(mzm,2,mean)
    bmi1     bmi2
22.68876 22.86700
cov(mzm)
         bmi1     bmi2
bmi1 7.240612 4.698384
bmi2 4.698384 6.921260
cor(mzm)
         bmi1      bmi2
bmi1 1.0000000 0.6636946
bmi2 0.6636946 1.0000000
```

```
apply(dzm,2,mean)
    bmi1     bmi2
23.32167 23.16760
cov(dzm)
         bmi1     bmi2
bmi1 9.208189 1.574196
bmi2 1.574196 5.799376
cor(dzm)
         bmi1      bmi2
bmi1 1.0000000 0.2154175
bmi2 0.2154175 1.0000000
```

Scatter plots of male and female twins

```
jpeg("ACE.jpg")
par(mfrow=c(2,2))
plot(mzm)
plot(dzm)
plot(mzw)
plot(dzw)
dev.off()
```

BMIs in MZ twins are seen to be more correlated than those in DZ twins.



Structural equation modelling

- Multiple and multivariate regression
- Confirmatory factor analysis
- Latent growth curves
- Latent differential equations
- Moderated parameter models
- Multigroup models
- Multilevel multivariate models with moderated parameters

Mx, MxGUI, OpenMx

- Mx is a well-established software for structural equation modeling and in particular widely used in twin modeling.
- MxGUI is Windows-based program which greatly facilitate the modeling process.
- OpenMx is a recent initiative to take advantage of the R environment. It carries over a variety of features from Mx/MxGUI.
- As it implies, the software is freely available from http://openmx.psyc.virginia.edu
- As it is written in R, it is considerably simple in our context to explore functionality it provides. We have adapted some examples from OpenMx website and for extended description, it is recommended to visit there.

Mx specification for twin data I

```
G1: model parameters
Data Calc NGroups=4
Begin Matrices;
X Lower 1 1 Free
Y Lower 1 1 Free
Z Lower 1 1 Free
W Lower 1 1 Fixed
Begin Algebra;
A= X*X' ;
C= Y*Y' ;
E= Z*Z' ;
D= W*W' ;
End Algebra;
End
```

```
G2: MZ
Data NInput-vars=2 NObservations=522
Labels N_t1 N_t2
CMatrix
.73865671
.68574888 1.3722835
Matrices= Group 1
Covariances  A+C+D+E | A+C+D _
    A+C+D   | A+C+D+E /
Options RSidual
End
```

Mx specification for twin data II

```
G3: Dizygotic twin pairs
Data NInput_vars=2 NObservations=272
Labels N_t1 N_t2
CMatrix
1.0942882
.3712542 .93089623
Matrices= Group 1
H Full 1 1
Q Full 1 1
Covariances A+C+D+E | H@A+C+Q@D _
            H@A+C+Q@D | A+C+D+E /
Matrix H .5
Matrix Q .25
Start .6 All
Options Multiple RSidual
End
```

```
G4: beta-test
data calc
matrices= Group 1
compute (A|C|D|E) @
    (A+C+D+E)~/
options rs nd=3
options multiple
end
```

OpenMx installation and a first session

```
source('http://openmx.psyc.virginia.edu/getOpenMx.R')
library(OpenMx)
?OpenMx

data(demoOneFactor)
head(demoOneFactor)

         x1         x2           x3          x4          x5
1 -0.1086832 -0.4669377 -0.177839881 -0.08093113 -0.07065026
2 -0.1464765 -0.2782619 -0.273882553 -0.15412007  0.09271729

vars <- names(demoOneFactor)
```

Model specification and fitting

```
Model <- mxModel("One Factor",
    type="RAM",
    manifestVars=vars,
    latentVars="G",
    mxPath(from="G", to=manifests),
    mxPath(from=vars, arrows=2),
    mxPath(from="G", arrows=2, free=FALSE, values=1.0),
    mxData(observed=cov(demoOneFactor), type="cov",
    numObs=500)
)
Fit <- mxRun(Model)
summary(Fit)
```

Parameter estimation

	name	matrix	row	col		Estimate	Std.Error
1	<NA>		A	x1	G	0.39715212	0.015549769
2	<NA>		A	x2	G	0.50366111	0.018232514
3	<NA>		A	x3	G	0.57724141	0.020448402
4	<NA>		A	x4	G	0.70277369	0.024011418
5	<NA>		A	x5	G	0.79624998	0.026669452
6	<NA>		S	x1	x1	0.04081419	0.002812717
7	<NA>		S	x2	x2	0.03801999	0.002805794
8	<NA>		S	x3	x3	0.04082718	0.003152308
9	<NA>		S	x4	x4	0.03938706	0.003408875
10	<NA>		S	x5	x5	0.03628712	0.003678561

Model-fitting statistics

observed statistics: 15
estimated parameters: 10
degrees of freedom: 5
-2 log likelihood: -3648.281
saturated -2 log likelihood: -3655.665
number of observations: 500
chi-square: 7.384002
p: 0.1936117
AIC (Mx): -2.615998
BIC (Mx): -11.84452
adjusted BIC:
RMSEA: 0.03088043

Elementary statements

We can obtain a list of commands as usual, i.e.,
library(help=OpenMx)

e.g.,
mxAlgebra
mxMatrix
mxData
mxEval
mxAlgebraObjective
mxFIMLObjective
mxRun

Examples

```
A <- mxMatrix("Full", nrow = 3, ncol = 3, values=2, name = "A")
A
FullMatrix 'A'

@labels: No labels assigned.

@values
     [,1] [,2] [,3]
[1,]    2    2    2
[2,]    2    2    2
[3,]    2    2    2

@free: No free parameters.

@lbound: No lower bounds assigned.

@ubound: No upper bounds assigned.
```

ACE model

```
ACE<-function(mzDat=mzData,dzDat=dzData,type="raw",selV=selVars){
twinACEModel <- mxModel("ACE",
mxMatrix("Full", 1, 1, TRUE, .6, "a", name="X"),
mxMatrix("Full", 1, 1, TRUE, .6, "c", name="Y"),
mxMatrix("Full", 1, 1, TRUE, .6, "e", name="Z"),
mxAlgebra(X %*% t(X), "A"), mxAlgebra(Y %*% t(Y), "C"),
mxAlgebra(Z %*% t(Z), "E"), mxAlgebra(A+C+E, name="V"),
mxMatrix("Full", 1, 2, TRUE, 20, "mean", name="expMean"),
mxAlgebra(rbind(cbind(A+C+E, A+C), cbind(A+C, A+C+E)), "expCovMZ"),
mxAlgebra(rbind(cbind(A+C+E, 0.5%x%A+C), cbind(0.5%x%A+C,
  A+C+E)), "expCovDZ"),
mxModel("MZ", mxData(mzDat, type), mxFIMLObjective("ACE.expCovMZ",
  "ACE.expMean", selV)),
mxModel("DZ", mxData(dzDat, type), mxFIMLObjective("ACE.expCovDZ",
  "ACE.expMean", selV)),
mxAlgebra(MZ.objective + DZ.objective, name="twin"),
mxAlgebraObjective("twin"))
```

Fitting ACE model

```
twinACEFit <- mxRun(twinACEModel, silent=TRUE)
exp_ACE <-
    mxEval(rbind(expCovMZ,expCovDZ,expMean),
    twinACEFit)
est_ACE <- mxEval(cbind(A,C,E,A/V,C/V,E/V), twinACEFit)
LL_ACE <- mxEval(objective, twinACEFit)
rownames(exp_ACE) <-
    c('CovMZT1','CovMZT2','CovDZT1','CovDZT2','Mean')
colnames(exp_ACE) <- c('T1','T2')
rownames(est_ACE) <- 'ACE'
colnames(est_ACE) <- c('a','c','e','a^2','c^2','e^2')
```

Fitting AE model

```
twinAEModel <- mxModel(twinACEModel, mxMatrix("Full",
    1, 1, FALSE, 0, "c", name="Y"))
twinAEFit <- mxRun(twinAEModel, silent=TRUE)
exp_AE <- mxEval(rbind(expCovMZ,expCovDZ,expMean),
    twinAEFit)
est_AE <- mxEval(cbind(A,C,E,A/V,C/V,E/V), twinAEFit)
rownames(est_AE) <- 'AE'
LL_AE <- mxEval(objective, twinAEFit)
LRT_ACE_AE <- LL_AE - LL_ACE
```

Fitting CE model

```
twinCEModel <- mxModel(twinACEModel, mxMatrix("Full",
    1, 1, FALSE, 0, "a", name="X"))
twinCEFit <- mxRun(twinCEModel, silent=TRUE)
exp_CE <- mxEval(rbind(expCovMZ,expCovDZ,expMean),
    twinCEFit)
est_CE <- mxEval(cbind(A,C,E,A/V,C/V,E/V), twinCEFit)
rownames(est_CE) <- 'CE'
LL_CE <- mxEval(objective, twinCEFit)
LRT_ACE_CE <- LL_CE - LL_ACE
```

Fitting E model and summary statistics

```
twinEModel <- mxModel(twinAEModel, mxMatrix("Full", 1, 1, FALSE, 0,
    "a", name="X"))
twinEFit <- mxRun(twinEModel, silent=TRUE)
exp_E <- mxEval(rbind(expCovMZ,expCovDZ,expMean), twinEFit)
est_E <- mxEval(cbind(A,C,E,A/V,C/V,E/V), twinEFit)
rownames(est_E) <- 'E'
LL_E <- mxEval(objective, twinEFit)
LRT_ACE_E <- LL_E - LL_ACE
exp <- cbind(exp_ACE,exp_AE,exp_CE,exp_E)
est <- rbind(est_ACE,est_AE,est_CE,est_E)
lls <-
    rbind(cbind(LL_ACE,0),cbind(LL_AE,LRT_ACE_AE),cbind(LL_CE,LRT_A
    CE_CE),cbind(LL_E,LRT_ACE_E))
df <- c(NA,1,1,2)
lls <- cbind(lls,pchisq(2*lls[,2],df,lower.tail=FALSE))
rownames(lls) <-
    c("l(ACE)","l(AE),lrt(AE,ACE)","l(CE),lrt(CE,ACE)","l(E),lrt(ACE,E)")
invisible(list(exp=exp,est=est,lls=lls))
}
```

Heritability estimates

The heritability and 95% boostrap CI estimates by models are as follows,

	mean	sd	lcl	ucl
ACE	0.6558460	0.04264874	0.5722545	0.7394376
AE	0.6579248	0.03891981	0.5816420	0.7342076
CE	0.0000000	0.00000000	0.0000000	0.0000000
E	0.0000000	0.00000000	0.0000000	0.0000000

This provides a simple estimation, although we could obtain analytical approximation in a more elaborate way.

Summary

- The pursuit of precise estimation of genetic vs environmental contributions to complex traits have a long history and currently an indispensible part of genetic epidemiology or statistical genetics.
- The literature we focused here is largely from 1970s onwards. However, we have gone quite far with our understanding and implementation of procedures. The former includes simple estimation, maximum likelihood methods, path analysis and structural equation modeling while the latter evolves from Fortran, LISREL/Mx/MxGUI to R.
- Our focus is on the practical side. The new practice with OpenMx rests on the flexible and powerful R computing environment, which makes collaborative work truly possible.

Information retrieval with NCBI2R

- Whenever there is a routine task, it calls for a formal programming. GWAS produces many p-values without full context and some annotation is needed.
- NCBI (http://www.ncbi.nlm.nih.gov/) is a good source with up-to-date information.
  - PubMed
  - All Databases
  - Books
  - OMIM
  - SNP
  - Taxomony
  - ...
- A direct access to the URL is convenient but tedious.
- A really simple solution is to use applications such as NCBI2R.

Setup

- As from v1.3, NCBI2R is available from CRAN and the project's homepage has more information: http://drop.io/NCBI2R_package.
- As usual the package is loaded into R as follows.

```
library(NCBI2R)
library(help=NCBI2R)
help.start()
```

- The package is still under development but most functions should work under the Windows environment.

OpenURL and GetPubMed

```
OpenURL("http://www.ncbi.nlm.nih.gov/")
refs <- GetPubMed("MC4R","MC4R.tab")
"Number of papers found in PubMed was: 594"
names(refs)
 [1] "PMID"      "TI"        "AB"        "OWN"       "STAT"      "DA"
 [7] "IS"        "DP"        "AU"        "LA"        "PT"        "DEP"
[13] "TA"        "JT"        "JID"       "EDAT"      "MHDA"      "CRDT"
[19] "AID"       "PST"       "SO"        "CI"        "AD"        "PHST"
[25] "VI"        "IP"        "PG"        "FAU"       "GR"        "PL"
[31] "SB"        "PMC"       "OID"       "MID"       "PMCR"      "DCOM"
[37] "LR"        "RN"        "MH"        "CIN"       "RF"        "SI"
[43] "TT"        "CN"        "EIN"       "IR"        "FIR"       "CON"
[49] "GN"        "OTO"       "OT"        "IRAD"      "localcopy" "link"

PrintFilters()
```

OpenPMID and OpenPDF

- We could use the following commands to save our query

  refs <- GetPubMed("MC4R")

  MakeExcel(refs,"MC4R.tab")
- We can browse the content

  fix(refs)
- We can examine papers in PDF format

  OpenPDF(refs$PMID[1])
- We can examine summary information as in PubMed

  OpenPmid(refs$PMID[1])

Annotation

This is achieved with functions available, e.g.,

ScanForGenes, ScanforSNPs

GetSNPInfo, GetGeneInfo

GetGeneInfo(#)

AnnotateDataframe(mydata, selections=c(“marker”,”p”,”beta”))

AnnotateSNPList, AnnotateSNPFile

GetIDs()

GetGeneTable(#)

GetGOs(#)

GetInteractions(#)

GetPathways(#)

GetRegion(“snp”,”4”,start,end), GetRegion(“gene”,”X”,start,end)

GetPhenotypes(#)

GetSNPsInGene(#)

Example: obesity-related SNPs

```
snps <- c("rs6548238",
  "rs7566605","rs745229","rs1106683","rs1121980","rs9
  939609","rs17782313","rs17700633")
snps_info <- GetSNPInfo(snps)
snps_split <- SplitGenes(snps_info)
snps_list <- AnnotateSNPList(snps,"snps.html")
snps_file <- AnnotateSNPFile("snps.txt","snps.html")
MakeExcel(snps_file,"snps.tab")
MakeHTML(snps_file,"snps.html")
```

GetSNPInfo, GetGeneInfo, GetNeighGenes

```
snps_info <- GetSNPInfo(snps)
snps_info
     marker genesymbol locusID chr     chrpos          fxn_class      species
 rs6548238                      2     624906                        Homo sapiens
 rs7566605                      2 118552496                        Homo sapiens
  rs745229   FAM71F1   84691    7 128146128 coding-synonymous,
                                                 reference          Homo sapiens
 rs1106683                      7 131104066                        Homo sapiens
 rs1121980       FTO   79068  16  52366749              intron      Homo sapiens
 rs9939609       FTO   79068  16  52378029              intron      Homo sapiens
rs17782313                     18  56002078                        Homo sapiens
rs17700633                     18  56080413                        Homo sapiens
GetNeighGenes("18",56002078,150000) # +/- 150Kbp
 chr LowPoint HighPoint      locusID
1  18 55852078  56152078 115701,23327
names(GetGeneInfo(23327))
 [1] "locusID"          "org_ref_taxname"    "org_ref_commonname"
 [4] "OMIM"             "synonyms"           "genesummary"
 [7] "genename"         "phenotypes"         "pathways"
[10] "GeneLowPoint"     "GeneHighPoint"      "ori"
[13] "chr"              "genesymbol"         "build"
[16] "cyto"             "approx"
```

Melanocortin 4 receptor (MC4R)

```
gid <- GetIDs("MC4R")
gnames <- GetGeneNames(gid)
ginfo <- GetGeneInfo(gid)
ggo <- GetGOs(gid)
gint <- GetInteractions(gid)
gpheno <- GetPhenotypes(gid)
gpath <- GetPathways(gid)
gsts <- GetUniSTSFromName("MC4R")
gstsinfo <- GetUniSTSInfo(gsts[1])
```

GetIDs and GetGeneNames

```
gid
  "4160" "342784" "79068" "100270981" "9709“ "2646“
    "400652" "1071" "4023" “181" "5443" "26033“ "4157"
    "132789" "9607" "89866" "9317" "23017“ "5566""4864"
    "4852" "4159"  "627" "4094“"434" "129787" "156"
names(gnames)
 "genename"  "genesymbol" "NewlocusID"
    "CurrentRecord" "LastUpdate" "locusID" "species"
gnames$genesymbol
  "MC4R" "LOC342784" "FTO" "RPL30P9" "HERPUD1"
    "GCKR“ "RPS3AP49" "CETP" "LPL" "AGRP" "POMC"
    "ATRNL1“ "MC1R" "GNPDA2" "CARTPT" "SEC16B"
    "PTER" "FAIM2“ "PRKACA" "NPC1" "NPY" "MC3R"
    "BDNF" "MAF“"ASIP" "TMEM18"  "ADRBK1"
```

GetGeneInfo and GetGeneTable

```
 names(ginfo)
 "locusID" "org_ref_taxname" "org_ref_commonname“
    "OMIM" "synonyms" "genesummary“ "genename"
    "phenotypes" "pathways“ "GeneLowPoint“
    "GeneHighPoint" "ori“ "chr" "genesymbol" "build“ "cyto"
    "approx"
 GetGeneTable(4160) # positions of exons, DNA/protein Acc #
$ExonInfo
   Where Start Stop Size Set
1    Exon     1 1438 1438   1
2 CodExon   420 1418  999   1
$ACC.DNA
   Identifier Length Exons
1 NM_005912.2   1438     1
$ACC.Prot
   Identifier Length Exons
1 NP_005903.2    332     1
```

ginfo[c("locusID","OMIM","chr","GeneLowPoint","GeneHighPoint","ori","genesymbol", "cyto")]

```
  4160 155541  18    58038564    58040001  -     MC4R       18q22
  342784       18    57863787    57865424  +            18q21.32
   79068 610966  16    53737875    54148381  +      FTO      16q12.2
100270981        8    19970847    19971168  -   RPL30P9       8p22
   9709 608070  16    56965748    56977793  +   HERPUD1   16q12.2-q13
   2646 600842   2    27719706    27746551  +     GCKR        2p23
  400652       18    57816776    57817639  +  RPS3AP49     18q21.32
   1071 118470  16    56995835    57017756  +     CETP       16q21
   4023 609708   8    19796582    19824770  +      LPL        8p22
    181 602311  16    67516474    67517716  -     AGRP       16q22
   5443 176830   2    25383722    25391559  -     POMC       2p23.3
  26033 612869  10   116853124   117708496  +    ATRNL1       10q26
   4157 155555  16    89984287    89987385  +     MC1R      16q24.3
 132789 613222   4    44704168    44728612  -    GNPDA2        4p12
   9607 602606   5    71014994    71016872  +    CARTPT       5q13.2
  89866 612855   1   177898242   177939050  -    SEC16B       1q25.2
   9317 604446  10    16478967    16555736  +     PTER       10p12
  23017 604306  12    50260680    50297720  -     FAIM2       12q13
   5566 601639  19    14202500    14228559  -    PRKACA      19p13.1
   4864 607623  18    21111463    21166470  -     NPC1     18q11-q12
   ...
```

GetGOs

ggo

category	name	evidence	pubmed	db	db_id
Function	G-protein coupled receptor activity	IEA		GO	4930
Function	melanocortin receptor activity	TAS	8794897	GO	4977
Function	protein binding	IEA		GO	5515
Function	receptor activity	IEA		GO	4872
Process	G-protein coupled receptor protein signaling pathway	IEA		GO	7186
Process	G-protein signaling, coupled to cAMP nucleotide second messenger	TAS	8794897	GO	7188
Process	feeding behavior	TAS	9771698	GO	7631
Process	insulin secretion	IEA		GO	30073
Process	regulation of bone resorption	IMP	16614075	GO	45780
Process	regulation of metabolic process	IEA		GO	19222
Process	response to insulin stimulus	IEA		GO	32868
Process	signal transduction	IEA		GO	7165
Component	cytoplasm	IDA	18029348	GO	5737
Component	integral to membrane	TAS	8392067	GO	16021
Component	plasma membrane	TAS	10585465	GO	5886

GetRegion, GetGeneInfo, GetSNPsInGene

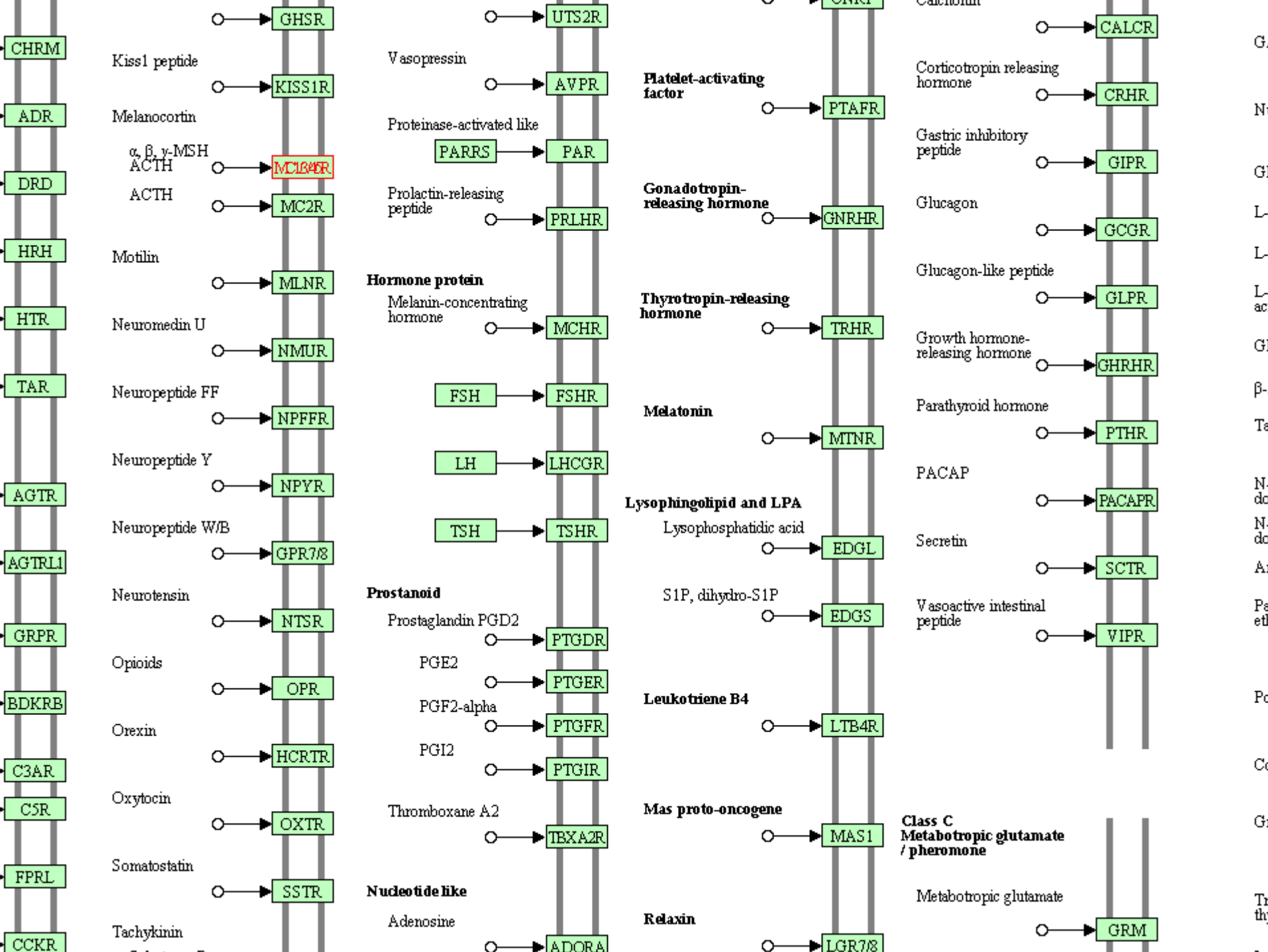
```
 GetRegion("snp","18",58038564,58040001)
"rs79783591" "rs79390404" "rs78877161" "rs76500026" "rs74679969"
"rs62097821" "rs61741819" "rs52820871" "rs52804924" "rs35351438"
"rs34114122" "rs13447340" "rs13447339" "rs13447338" "rs13447337"
"rs13447336" "rs13447335" "rs13447334" "rs13447333" "rs13447332"
"rs13447331" "rs13447330" "rs13447329" "rs13447328" "rs13447327"
"rs13447326" "rs13447325" "rs13447324" "rs13447323" "rs2282556"
"rs17848587" "rs52834737" "rs2229616"  "rs1016862“

 GetRegion("gene","18",58038564,58040001)
"4160"

GetGeneInfo(4160)
GetSNPsInGene(4160)
```

GetPathways

```
gpath
 name
1 KEGG pathway: Neuroactive ligand-receptor interaction
2                    Reactome Event:Signaling by GPCR
                                                  web
1          http://www.genome.jp/dbget-
   bin/show_pathway?hsa04080+4160
2 http://www.reactome.org/cgi-
   bin/eventbrowser_st_id?ST_ID=REACT_14797
```


CHRM
ADR
DRD
HRH
HTR
TAR
AGTR
AGTRL1
GRPR
BDKRB
C3AR
C5R
FPRL
CCKR
GHSR
Kiss1 peptide
KISS1R
Melanocortin
α, β, γ-MSH
ACTH
MC1R/4R
ACTH
MC2R
Motilin
MLNR
Neuromedin U
NMUR
Neuropeptide FF
NPFFR
Neuropeptide Y
NPYR
Neuropeptide W/B
GPR7/8
Neurotensin
NTSR
Opioids
OPR
Orexin
HCRTR
Oxytocin
OXTR
Somatostatin
SSTR
Tachykinin
UTS2R
Vasopressin
AVPR
Proteinase-activated like
PARRS
PAR
Prolactin-releasing peptide
PRLHR
Hormone protein
Melanin-concentrating hormone
MCHR
FSH
FSHR
LH
LHCGR
TSH
TSHR
Prostanoid
Prostaglandin PGD2
PTGDR
PGE2
PTGER
PGF2-alpha
PTGFR
PGI2
PTGIR
Thromboxane A2
TBXA2R
Nucleotide like
Adenosine
ADORA
Platelet-activating factor
PTAFR
Gonadotropin-releasing hormone
GNRHR
Thyrotropin-releasing hormone
TRHR
Melatonin
MTNR
Lysophingolipid and LPA
Lysophosphatidic acid
EDGL
S1P, dihydro-S1P
EDGS
Leukotriene B4
LTB4R
Mas proto-oncogene
MAS1
Relaxin
LGR7/8
CALCR
Corticotropin releasing hormone
CRHR
Gastric inhibitory peptide
GIPR
Glucagon
GCGR
Glucagon-like peptide
GLPR
Growth hormone-releasing hormone
GHRHR
Parathyroid hormone
PTHR
PACAP
PACAPR
Secretin
SCTR
Vasoactive intestinal peptide
VIPR
Class C Metabotropic glutamate / pheromone
Metabotropic glutamate
GRM

Entry	4160 CDS H. sapiens
Gene name	MC4R
Definition	melanocortin 4 receptor
Orthology	KO: K04202 melanocortin 4 receptor
Pathway	PATH: hsa04080 Neuroactive ligand-receptor interaction
Class	Environmental Information Processing; Signaling Molecules and Interaction; Neuroactive ligand-receptor interaction [PATH: hsa04080] BRITE hierarchy
SSDB	Ortholog Paralog Gene cluster GFIT
Motif	Pfam: 7TM_GPCR_Srv 7TM_GPCR_Srh 7TM_GPCR_Srw 7TM_GPCR_Srx 7TM_GPCR_Srsx 7tm_1 TAS2R Colicin_im PROSITE: G_PROTEIN_RECEP_F1_1 G_PROTEIN_RECEP_F1_2 Motif
Other DBs	OMIM: 155541 NCBI-GI: 119508433 NCBI-GeneID: 4160 HGNC: 6932 HPRD: 01116 Ensembl: ENSG00000166603 UniProt: B2RAC3 P32245

Position	18q22
AA seq	332 aa AA seq DB search MVNSTHRGMHTSLHLWNRSSYRLHSNASESLGKGYSDGGCYEQLFVSPEVFVTLGVISLL ENILVIVAIAKNKNLHSPMYFFICSLAVADMLVSVSNGSETIVITLLNSTDTDAQSFTVN IDNVIDSVICSSLLASICSLLSIAVDRYFTIFYALQYHNIMTVKRVGIIISCIWAACTVS GILFIIYSDSSAVIICLITMFFTMLALMASLYVHMFLMARLHIKRIAVLPGTGAIRQGAN MKGAITLTILIGVFVVCWAPFFLHLIFYISCPQNPYCVCFMSHFNLYLILIMCNSIIDPL IYALRSQELRKTFKEIICCYPLGGLCDLSSRY
NT seq	999 nt NT seq atggtgaactccacccaccgtgggatgcacacttctctgcacctctggaaccgcagcagt tacagactgcacagcaatgccagtgagtcccttggaaaaggctactctgatggagggtgc tacgagcaacttttgtctctcctgaggtgtttgtgactctgggtgtcatcagcttgttg gagaatatcttagtgattgtggcaatagccaagaacaagaatctgcattcacccatgtac tttttcatctgcagcttggctgtggctgatatgctggtgagcgtttcaaatggatcagaa accattgtcatcaccctattaaacagtacagatacggatgcacagagtttcacagtgaat attgataatgtcattgactcggtgatctgtagctccttgcttgcatccatttgcagcctg ctttcaattgcagtggacaggtactttactatcttctatgctctccagtaccataacatt atgacagttaagcgggttgggatcatcataagttgtatctgggcagcttgcacggtttca ggcattttgttcatcatttactcagatagtagtgctgtcatcatctgcctcatcaccatg ttcttcaccatgctggctctcatggcttctctctatgtccacatgttcctgatggccagg cttcacattaagaggattgctgtcctccccggcactggtgccatccgccaaggtgccaat atgaagggagcgattaccttgaccatcctgattggcgtctttgttgtctgctgggcccca ttcttcctccacttaatattctacatctcttgtcctcagaatccatattgtgtgtgcttc atgtctcactttaacttgtatctcatactgatcatgtgtaattcaatcatcgatcctctg atttatgcactccggagtcaagaactgaggaaaaccttcaaagagatcatctgttgctat cccctgggaggcctttgtgacttgtctagcagatattaa

Obesity and MC4R

```
oid <- GetIDs("obesity[MC4R]")
oinfo <- GetGeneInfo(oid)
names(oinfo)
 [1] "locusID"          "Org_ref_taxname"
    "Org_ref_commonname" "OMIM"              "synonyms"
 [6] "genesummary"      "genename"          "phenotypes"
    "phenotypes.HTML"   "pathways"
[11] "pathways.HTML"    "GeneLowPoint"      "GeneHighPoint"
    "Ori"              "Chromosome"
[16] "genesymbol"       "build"             "cyto"
    "approx"
dim(oinfo)
302 19
```

A tutorial example

```
favouriteSNP <- "rs4294787"
favouriteSNPInfo <- GetSNPInfo(favouriteSNP)
pathway <- GetPathways(favouriteSNPInfo$locusID)
genes_in_pathway <- GetIDs(pathway$name)
#a loop to enable GetSNPsInGene work with multiple genes
for (i in 1:length(genes_in_pathway)) {
   if(!(exists("biglist"))) {
      biglist <- GetSNPsInGene(genes_in_pathway[i])
   } else {
     biglist<-c(biglist,GetSNPsInGene(genes_in_pathway[i]))
   }
}
length(biglist)
165212
```

Side interest

```
# An example showing the principle as implemented in the
   package can be useful for obtaining other information.
 keywords <- c("Professor","England")
 nj <- NatureJobs(keywords,"nj",days=7)
 dim(nj)
120 11
 names(nj)
 names(nj)
 [1] "JobTitle"       "Employer"       "Location"       "Posted"
   "Desc"          "DaysAgo"       "IDnumber"
   "BigDescription"
 [9] "WebLink"        "LocalLink"      "ExpDate"
```

A summary

- NCBI, especially PubMed, has been a major source of biomedical information retrieval in daily research. The process can considerably be facilitated by R/NCBI2R package. A minor issue is that it only retrieves the latest information but earlier information is very useful (e.g., build 35). Hence more experiences need to be gathered.
- "NCBI2R annotates lists of SNPs and/or genes, with current information from NCBI … designed to allow those performing the genome analysis to produce output that could easily be understood by a person not familiar with R". It is easy to anticipate that more functionality can be added from the same principle. It is helpful to keep an eye on the package development even if implementation may not necessarily be a priority in our research.

Further information

- We often use NCSC genome browser (http://genome.ucsc.edu) coupled with the galaxy system (http://g2.bx.psu.edu), but the facility in R will be complementary.
- Annotation databases (Lesk AM. Database Annotation in Molecular Biology-Principles and Practice. Wiley 2005)
- Other packages such as gene2pathway from CRAN (Prediction of KEGG pathway membership for individual genes based on InterPro domain signatures), SubpathwayMiner (Annotation and identification of the KEGG pathways)
- Packages from BioConductor (http://www.bioconductor.org), e.g., KEGGSOAP (interface to the KEGG SOAP server)
  - library(annotate)
  - ab <- pubmed[“18454148”]
  - buildPubMedAbst(xmlRoot(ab)[[1]])
  - pubmed(“18454148”, disp=“browser”)

RNCBI

- library(RNCBI)
- ncbi <- NCBI()
- einfo <- EInfo(ncbi)
- einfo <- setRequestParameter(einfo, "db", "pubmed")

V Conclusion

General comments

- As has been driven by technological advances in genotyping and computational technology, the genetic analysis of complex trait is a dynamic topic in a fast-moving field.
- The R environment is now indispensible with a great deal of recognition and stability. Nevertheless, there are areas which can be further advanced, e.g., graphics. A range of models available from R remains to be explored and have been supplementary to the main analysis.

Scientific aspects of GWAS

- What are the uses?
  - Discovery of new susceptibility loci
  - Elucidate biologic pathways
  - Identify links between these loci and covariates
  - Risk prediction
- Where would they go?
  - Expanding well-characterized study populations
  - Expanding the range of genetic variation including structural variants and lower-frequency common variants
  - Documenting functional mechanisms responsible for the association signals.

Chanock (*Personal Communication*) and Altshuler et al. *Science* 2008, 322:881-8

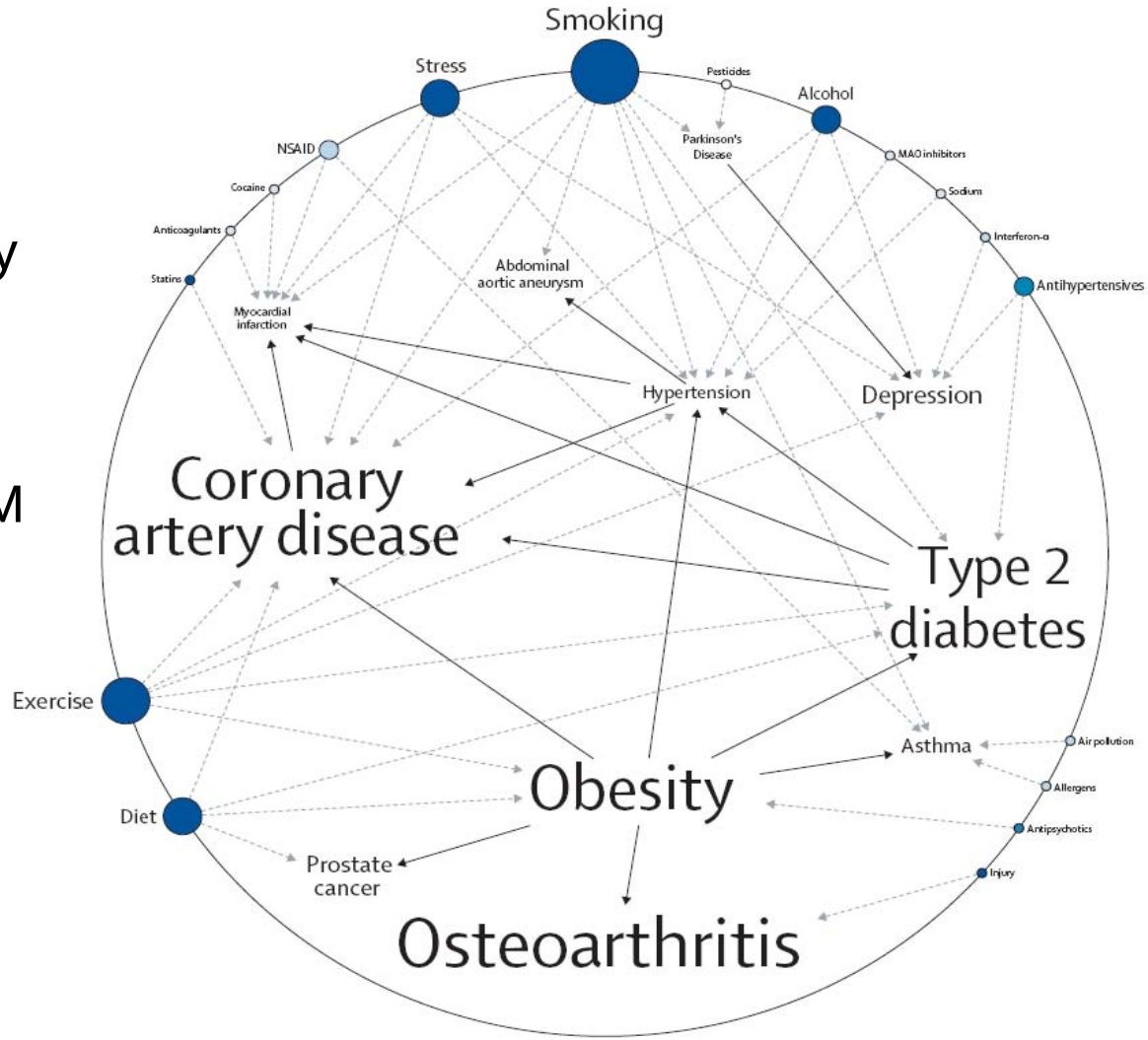
Limitations and practical issues

- Limitations
  - It requires large sample sizes
  - It only identifies loci, not genes
  - It detects only common alleles in a population
  - It usually does not go into the expression level
- Practical issues
  - For individual studies, the issues as of epidemiological studies in general remain and there is uncertainty to declare statistical significance
  - For consortium meta-analysis, there may be difference in quality control, data sharing and variation in complexity of analysis
- Technological advances, e.g., sequencing, remain to have profound influences.

A great expectation

Ashley *et al. Lancet* 2010, 375, 1525-35

The authors assessed a patient with a family history of vascular disease and early sudden death. The analysis involved 2.6M SNPs and 752 CNVs showing increased genetic risk for MI, T2D and some cancers.



Summary

- The need from various analyses seeds the development in R and shares much in common with many other problems involving large data, such as interactive graphics in combination with publicly available databases, the use of statistical and computational facilities available from the R system.
- Applications in substantive areas are the constant source of motivation in package development. The implementation is likely to be patchy but with a great prospect, e.g., advanced models and causal pathways.
- Alternative computing environments are complementary.

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